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The Association between Caries and Periodontal Diseases

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The Association between Caries and Periodontal Diseases

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Résumé

Objectifs: Le but de cette étude clinique était de comparer un groupe d'adultes ayant un parodonte sain avec un groupe d'adultes atteints de parodontite chronique en terme de risque carieux et mesures cliniques et microbiologiques de la carie.

Méthodes: Quatre-vingt-seize individus ont été divisés en deux groupes en fonction de leur état de santé parodontal et ont été appariés pour l'âge, le sexe et l'origine ethnique. Trente-huit sujets étaient atteints de parodontite chronique définie comme ayant au moins quatre dents avec ≥ 1 site avec une profondeur de sondage ≥ 4 mm et une perte d'attache clinique ≥ 2 mm, et 58 sujets présentaient un parodonte sain. Par la suite, les groupes ont été subdivisés en deux groupes en fonction de leur statut carieux : les participants ayant au moins une lésion carieuse non traitée sur une surface dentaire et ceux n'ayant pas de lésion carieuse non traitée. Les données ont été recueillies par le biais d'un questionnaire, un examen clinique et des échantillons de plaque supra- et sous-gingivale. L'évaluation de la charge buccale de *Streptococcus mutans* et de six agents pathogènes parodontaux a été réalisée par la technique d'amplification de la réaction en chaine de la polymérase (PCR). Les données ont été analysées à l'aide d'analyses statistiques descriptives et bivariées.

Résultats: Les individus atteints de parodontite chronique étaient 3,5 fois plus susceptibles d'avoir des caries que les individus en bonne santé (OR 3,5 ; IC: 1,5 - 8,3 ; *P* = 0,006). Les sujets à la fois atteints de parodontite chronique et de caries dentaires ont eu un niveau d'éducation significativement plus faible que les sujets ayant un parodonte sain et sans caries dentaires (OR 6,0 ; IC: 1,7 à 21,7 ; *P* = 0,04). La proportion de sujets ayant une charge buccale élevée de *Porphyromonas gingivalis* (*P. g.*) et *Treponema denticola* (*T. d.*) était significativement plus élevée chez les patients atteints de parodontite chronique et de carie que chez les patients sains présentant des caries (*P. g.:* OR 8,6 ; IC: 2,4 - 30,3 ; *P* = 0,004 et *T. d.*: OR 10,0 ; CI: 2,6 - 38.1 ; *P* = 0,003).

Conclusions: Les résultats de cette étude suggèrent que, chez les sujets adultes atteints de la parodontite chronique, la fréquence des caries est plus élevée que chez les sujets ayant un parodonte sain. De plus, le faible niveau d'éducation influence négativement le statut parodontal des individus.

Mots-clés: Maladie parodontale, caries, *Streptococcus mutans*, *Porphyromonas gingivalis*, *Treponema denticola*

Abstract

Aim: The aim of this clinical study was to compare adults with a healthy periodontium and those with chronic periodontitis, in terms of caries' risk and caries' clinical and microbiological measures.

Methods: Ninety-six healthy adults were divided into chronic periodontitis (n= 38) and healthy periodontium (n=58) based on their periodontal status, and matched for age, gender, and ethnic background. Chronic periodontitis was defined as having at least four teeth with ≥1 site with a pocket depth ≥4 mm and clinical attachment loss ≥2 mm. Each group were subsequently subdivided in 2 groups according to their caries status: participants having at least one untreated decayed surface and those with no untreated caries. Data were collected by means of self-administrated questionnaire, clinical examination, and supra- and subgingival plaque sampling. Assessments of oral levels of *Streptococcus mutans* and six periodontal pathogens were conducted by PCR amplification techniques. Data were analyzed using descriptive and bivariate statistical tests.

Results: Individuals with chronic periodontitis were 3.5 times more likely to have caries than healthy individuals (OR 3.5; CI: 1.5 – 8.3; $P = 0.006$). Subjects with both chronic periodontitis and dental caries had a significantly lower level of education than periodontally healthy subjects without dental caries (OR 6.0; CI: 1.7 – 21.7; $P = 0.04$). A significant higher proportion of subjects with high oral levels of *Porphyromonas gingivalis (P. g.)* and *Treponema denticola (T. d.)* was found among subjects with chronic periodontitis and untreated caries compared to periodontally healthy subjects with untreated caries (*P. g.*: OR 8.6; CI: 2.4 – 30.3; $P = 0.004$ and *T. d.*: OR 10.0; CI: 2.6 – 38.1; $P = 0.003$).

Conclusion: The results from this study suggest that, adults with chronic periodontitis are more prone to caries disease than those adults with a healthy periodontium. Furthermore, low educational level could have a negative impact on the periodontal status of individuals.

Keywords: periodontal disease, caries, *Streptococcus mutans*, *Porphyromonas gingivalis*, *Treponema denticola*

Table of Contents

List of Tables

Chapter 1

Chapter 2

Chapter 3

List of Figures

Chapter 1

Chapter 2

Chapter 3

List of Acronyms and Abbreviations

°	Degree
mm	Millimeter
mM	Millimolar
µl	Microliter
mg	Milligram
g	Gram
DMFS	Decayed, Missing and Filled tooth surfaces index
DMFT	Decayed, Missing and Filled teeth index
BOP	Bleeding on probing
PD	Pocket depth
PI	Plaque index
CAL	Clinical attachment loss
P. g.	*Porphyromonas gingivalis*
T. d.	*Treponema denticola*
P. i.	*Prevotella intermedia*
A. a.	*Aggregatibacter actinomycetemcomitans*
S. m.	*Streptococcus mutans*
T.f.	*Tannerella forsythia*
CI	Confidence interval
OR	Odds ratio
SD	Standard deviation
P	*P* value
PCR	Polymerase chain reaction
dNTP	Deoxyribonucleotide triphosphate
AAP	American Academy of Periodontology
CHMS	Canadian Health Measure System
OD	Optical density

I dedicate this work to my family for all the loving support during my life

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CHAPTER ONE

Introduction

and

Literature Review

1: Introduction

Poor oral health is an important determinant of morbidity and mortality in the population at large (World Health Organization., 1987; Appollonio et al., 1997; Shimazaki et al., 2001). There is also growing evidence of the association between oral and general health. Periodontal diseases have been shown to be implicated in the causal pathway of diabetes, coronary heart disease, and premature birth, among other conditions (Chambrone et al., 2011; Lakschevitz et al., 2011; Manjunath et al., 2011). Unfortunately, oral diseases such as dental caries and periodontal diseases are among the most prevalent chronic diseases in Canada and worldwide (Ong, 1998; Beltran-Aguilar and Beltran-Neira, 2004; Sanz and van Winkelhoff, 2011).

A recent Canadian Health Measures Survey (CHMS) found that 57% of children aged 6–11 years, 59% of 12–19-year-olds, 96% of adults, and 21% of dentate Canadian adults have moderate to severe periodontal disease (Health Canada, 2010). Furthermore, 96% of adults reported history of dental caries (Health Canada, 2010). Epidemiologic data from the province of Québec demonstrated that a majority of adult Quebecers suffer from periodontal disease and almost half of their tooth surfaces are affected by caries (Brodeur et al., 2000; Brodeur et al., 2001).

These data indicate that the Canadian oral healthcare system faces major challenges. In 2009, Canada spent 12.8 billion dollars on oral health care (Health Canada, 2010). Thus, there is a need to identify risk factors of poor oral health that could be improved by preventive measures. However, clinical studies examining the interaction of oral diseases, their common ethological pathogens, risk factors, and microbiological interactions are scarce and need to be addressed.

This research project investigating the association between dental caries and periodontal diseases in Quebec adults was therefore conducted to provide new evidence to further our understanding of whether exposure to periodontal pathogens will influence the susceptibility of individuals to dental caries. This chapter consists of a review of the literature offering background knowledge on this topic.

1.1: Periodontal Disease

1.1.1: Definition and Classification

Periodontal disease is defined as “an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament, alveolar bone loss, pocket formation, and gingival recession” (Novak, 2012).

The classification of periodontal diseases has been modified over the years. In 1989, the “World Workshop on Clinical Periodontics” proposed classification of different forms of periodontal disease including early-onset periodontitis, adult periodontitis, necrotizing ulcerative periodontitis, refractory periodontitis, and periodontitis associated with systemic diseases (Nevins et al., 1989; Novak, 2012).

Ten years later, a different classification of periodontal diseases was reported by the “International Workshop for a Classification of Periodontal Diseases and Conditions” and has been endorsed by the American Academy of Periodontology (International Workshop for a Classification of Periodontal Diseases and Conditions, 1999) (Table 1).

The most prevalent form of periodontal disease is chronic periodontitis, formerly known as adult periodontitis. The term “chronic” was selected because it was less limited in being neither specific nor age-dependent (International Workshop for a Classification of Periodontal Diseases and Conditions, 1999; Armitage, 1999). The 1999 International Workshop defined chronic periodontal disease as “an infectious disease resulting in inflammation within the supporting structures of teeth, and progressive attachment and bone loss. It is characterized by pocket formation and/or gingival recession. Its onset may be at any age but is most commonly detected in adults. The prevalence and the severity of the disease increase with age. It may affect a variable number of teeth and has variable rates of progression.”(Armitage, 1999). Moreover, the amount of destruction is in line with the presence of local factors, and signs of inflammation are variable according to the patient’s plaque control (International Workshop for a Classification of Periodontal Diseases and Conditions, 1999).

Depending on the number of sites involved, chronic periodontitis can be either localized or generalized. Periodontitis is classified as localized periodontitis when 30% or less of sites are affected, and as generalized periodontitis if more than 30% of sites are affected (International Workshop for a Classification of Periodontal Diseases and Conditions, 1999).

Table 1.1: Abbreviated Version of the 1999 AAP Classification of Periodontal Diseases and Conditions

Gingival Disease • Dental Plaque-Induced Gingival Diseases • Non-Plaque-Induced Gingival Diseases
Chronic Periodontitis • Localized • Generalized (More than 30% of sites are involved)
Aggressive Periodontitis • Localized • Generalized (Interproximal attachment loss affecting at least three permanent teeth other than first molars and incisors)
Periodontitis as a Manifestation of Systemic Diseases • Associated with Hematological Disorders • Associated with Genetic Disorders • Not Otherwise Specified
Necrotizing Periodontal Diseases • Necrotizing Ulcerative Gingivitis • Necrotizing Ulcerative Periodontitis
Abscesses of the Periodontium • Gingival Abscess • Periodontal Abscess • Pericoronal Abscess
Developmental or Acquired Deformities and Conditions • Localized Tooth-related Factors that Modify or Predispose to Plaque-induced Gingival Disease/Periodontitis • Mucogingival Deformities and Conditions Around Teeth • Mucogingival Deformities and Conditions on Edentulous Ridges

1.1.2: Epidemiology

Periodontal disease can affect up to 90% of the population, depending on race, geographic location, and diagnostic threshold (Pihlstrom et al., 2005). Mild to moderate chronic periodontitis is the most common form of periodontitis worldwide, with prevalence ranging from 13% to 57% depending on oral hygiene and socio-economic status (Rylev and Kilian, 2008). According to the 2007-2009 Canadian Health Measure Center survey (CHMS), 32.3% of Canadian adults showed signs of gingivitis in one or more locations in the mouth (Health Canada, 2010). In addition, 21% of adults Canadian demonstrated a moderate or severe form of periodontal disease (Health Canada, 2010). Moreover, 21.1% of Canadians had lost 4 mm or more of attachment and 6.0% had lost 6 mm or more (Health Canada, 2010). In addition, periodontitis is one of the major causes of tooth loss among the Quebec population and about half of 35-to-44-year-old Quebecers have at least one tooth with a periodontal pocket of 4 to 5 mm and one person out of five has at least one periodontal pocket >6 mm (Brodeur et al., 2001). Moreover, it was estimated that just 5.2% of Quebec adults do not need any periodontal therapy (Brodeur et al., 2001).

1.1.3: Etiology

The nature and the quantity of the oral microflora differ between sites within a patient and among patients (Moore et al., 1984). The oral biofilm contains mainly microbes and host proteins that start to adhere to teeth within a few minutes following dental oral hygiene procedures. Equal proportions of gram-positive cocci, especially *streptococcus spp* and *actinomyces sp*., dominate in the healthy gingival sulcus (Abiko et al., 2010; Darveau, 2010). Maturing plaque contributes to the development of facultative anaerobic microorganisms, spirochaetes, and motile rods. With increased disease severity, the strict proportions of anaerobic, gram-negative, and motile organisms increase significantly (Zambon, 1996; Sakamoto et al., 2005).

Transition from gingivitis to periodontitis is not well understood but it may be explained by the implication of host defense mechanisms, additional microbial species, and the invasion of certain

species into the periodontal tissue (Modeer and Wondimu, 2000). Investigators have underlined the fact that there are more than 500 species of microorganisms, such as gram-positive and gram-negative bacteria, yeast, protozoa, and viruses inhabiting the oral cavity, and their interactions within the biofilm are yet to be understood (Moore and Moore, 1994; Socransky and Haffajee, 1994; Paster et al., 2001). Anaerobic gram-negative rods and spirochetes are among the most prevalent bacteria in the periodontal lesion (Holt and Ebersole, 2005; Zijnge et al., 2012). The predominant species include *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia* formerly known as *Bacteroides forsythus*, *Aggregatibacter actinomycetemcomitans*, *Veillonella parvula*, *Wollinella recta*, *Eikenella corrodens*, *Treponema denticola, Peptostreptococcus micros*, *Prevotella melaninogenica*, *Fusobacterium nucleatum*, *Campylobacter rectus*, *Selenomonas sputigena*, and *Streptococcus intermedius* (Lovegrove, 2004; Holt and Ebersole, 2005; Zijnge et al., 2012). Most of these bacteria are highly motile and proteolytic. Some studies indicated that among these bacteria, *A. actinomycetemcomitans, P. gingivalis,* and *T. forsythia* are highly related to destruction of the periodontal tissues (Sanz et al., 2004). On the other hand, colonization of these bacteria has been reported to vary among populations with different race/ethnicity or geographic origins (Sanz et al., 2000; Haffajee et al., 2004; Lopez et al., 2004). For instance, Sanz et al. in 2000 studied subgingival plaque samples of people living in Spain and the Netherlands and matched the groups for age, gender, and periodontal disease variables (Sanz et al., 2000). They observed that samples of Spaniards exhibited high oral levels of *P. gingivalis* and low levels of *A. actinomycetemcomitans*, while Dutch samples demonstrated high prevalence of *A. actinomycetemcomitans* and *P. micros*. Griffen et al. studied patients of The Ohio State University College of Dentistry, and showed that there was a difference in the level of *P. gingivalis* in healthy periodontium depending on ethnic background, being positive in 22% of whites, 53% of African-Americans, and 60% of Asian-Americans (Griffen et al., 1998). *A. actinomycetemcomitans* is mostly associated with aggressive periodontitis in young adolescents (Henderson et al., 2010). *P. gingivalis* is a gram-negative black-pigmenting anaerobic bacterium, playing a key role in the initiation and progresssion of chronic periodontal disease (Loesche, 1999; Grenier and La, 2011). Furthermore, a cross-sectional study on 1,300 individuals indicated that *P. gingivalis* and *Tannerella forsythia* are associated with attachment loss or alveolar bone loss (Grossi et al., 1994; Grossi et al., 1995). In 1998, Socransky and colleagues using DNA probes identified that *T. forsythia, P. gingivalis,* and *T. denticola* were associated with increasing pocket depth and bleeding on probing (Socransky et al., 1998). A variety of other local factors including oral hygiene, pH, temperature, availability of nutrients and

water, anatomy of oral structures, salivary flow, and use of chemotherapeutic agents have been also reported to influence the growth of oral microorganisms (Marcotte and Lavoie, 1998; Burt, 2005; Pihlstrom et al., 2005).

1.1.4: Pathologic Pathways

Microorganisms inhabiting the oral biofilm, such as Gram anaerobes, can release endotoxins that penetrate periodontal tissues and trigger a host immune response. This response can result in tissue destruction either directly, by the action of enzymes and endotoxins, or indirectly by stimulating an inflammatory reaction within the host tissues through different pathways (Somma et al., 2010). Tissue response to bacterial antigens can be both protective and destructive. For example, bacterial toxins can stimulate the immune system to overproduce cytokines. Although inflammatory mediators such as cytokines are usually beneficial for tissue healing, in high quantity they can result in inflammation and tissue damage. The immune response to bacteria results in stimulating the periodontal tissue host cells to release enzymes such as matrix metalloproteinase that breaks down the connective tissue, leading ultimately to tooth loss. These enzymes and inflammatory mediators can not only affect the tissues of the oral cavity, but also can potentially damage other body organs by entering into the vascular system (Pender et al., 1997; Graves and Cochran, 2003).

Chronic periodontitis often results in gingival recession, which exposes the root surface to the aerobic flora of the mouth. The saliva buffering action can neutralize the pH level on the root surface and change the environmental condition into one that enables the growth of cariogenic bacterial species on the root surface (Saotome et al., 2006). This phenomenon can explain the potential risk of root caries in patients affected with chronic periodontitis, since the root surface is more susceptible to caries than the enamel of the tooth crown.

1.1.5: Risk Factors

Risk factor is defined as “an environmental exposure, aspect of behavior or an inherent characteristic which is associated with a disease” (Last and Association internationale d'épidémiologie, 2001). Different biological, behavioral, and systemic conditions have been introduced as potential risk factors for periodontal disease. These include age, gender, race/ethnicity, genetics, socioeconomic status, smoking, diabetes, obesity, HIV infection, osteoporosis, and psychological factors (Borrell and Papapanou, 2005).

Age

Although severe periodontitis can also be found in young adults, the severity and prevalence of periodontal disease increases with age (Burt, 1992; Borrell and Papapanou, 2005; Burt, 2005; Pihlstrom et al., 2005). It is hypothesized that degenerative systemic changes as a result of age may increase the susceptibility of elders to this chronic disease. The level of attachment loss increases with age, while pocket depth variation is minimal over the years (Albandar, 2002). Attachment loss and bone loss in elders might be caused by prolonged exposure to other risk factors during the individual’s life. Conversely, studies have demonstrated that elders following a specific preventive program can have minimal attachment loss (Persson et al., 1998).

Gender

Although, there is no inherent difference between men and women in susceptibility to periodontal disease, this disease is more prevalent in men. This might be explained by the fact that women pay usually more attention to their oral hygiene and use dental services more often (Albandar, 2002; Burt and Eklund, 2005). Therefore, gender differences observed in the prevalence and severity of periodontal diseases can be a result of a higher frequency of preventive methods rather than genetics.

Race/Ethnicity

National surveys in the United States have demonstrated that ethnicity is an important risk factor for periodontal disease (Albandar et al., 1997; Albandar et al., 1999). Periodontal disease has been seen to

vary significantly among different populations as a result of different levels of exposure to various etiological and risk factors of periodontitis in addition to differences in genetic profiles (Vo and Park, 2008; Sanders Thompson et al., 2009). Numerous studies have reported a higher prevalence of periodontal diseases in African-Americans and a similar prevalence in Mexicans and non-Hispanic white people (Borrell et al., 2002; Hyman and Reid, 2003). Similarly, Borrell *et al* found Black individuals to demonstrate twice as often periodontal disease versus Caucasian subjects (Borrell et al., 2005). Moreover, specific periodontal pathogens are seen in certain racial and ethnic populations. For example, *Aggregatibacter actinomycetemcomitans* serotype c colonizes more often Asian populations while the JP2 clone of *A. actinomycetemcomitans periodontitis* causes aggressive periodontitis in adolescents of the Mediterranean and Western parts of Africa (Haubek et al., 2001; Kim et al., 2009).

Genetic factors

Genetic factors play a significant etiologic role in about half of the subjects affected by periodontal diseases (Michalowicz, 1994). Studies on twins have demonstrated that clinical measures of periodontitis such as probing pocket depth and attachment loss can be affected by genetic factors. The family history of aggressive periodontitis indicates the genetic involvement of this category of periodontal disease (Loos et al., 2005; Frydman and Simonian, 2011). Although the most important modifier of periodontal phenotypes is genetic determination, the role of single nucleotide polymorphisms is ambiguous (Hart and Kornman, 1997; Schenkein, 2002).

Socio-economic status

Lower socio-economic status does not alone result in increased prevalence of periodontal diseases when adjusted for other factors such as smoking and poor oral hygiene (Novak, 2012). However, periodontal disease is more prevalent in low socio-economic populations (Lopez et al., 2001). This might be due to reduced accessibility to oral health facilities.

Smoking

The prevalence of periodontal disease, gingival recession, and attachment loss is higher in smokers than in non-smokers (Barbour et al., 1997; Albandar et al., 2000), and there is a strong association between smoking habit and both prevalence and severity of periodontal disease (Albandar et al., 2000; Bergstrom et al., 2000). Longitudinal clinical studies have reported that the influence of smoking has a

greater impact on the healing process of periodontal disease than on disease progression (Kinane and Radvar, 1997; Faddy et al., 2000; Papantonopoulos, 2004; Rieder et al., 2004).

Diabetes mellitus

Over the last decades, diabetes mellitus has been considered as one of the major risk factors for periodontitis (Grossi and Genco, 1998; Lalla et al., 2008). In fact, a two-way relationship between diabetes mellitus and periodontal diseases has been reported (Soskolne and Klinger, 2001). Subjects with diabetes mellitus have higher prevalence and severity of periodontal disease (Taylor et al., 1998; Lalla et al., 2008). Moreover, individuals with controlled diabetes have exhibited good response to treatment of periodontitis compared to poorly controlled diabetes subjects (Tervonen et al., 1991; Christgau et al., 1998; Mealey and Oates, 2006).

Obesity

In clinical studies, a positive relationship between obesity (body mass index [BMI] $\geq$ 30) and periodontitis has been reported (Saito et al., 2001; Al-Zahrani et al., 2003; Wood et al., 2003). It has been found that overweight subjects with insulin resistance have 1.5 times more periodontitis than individuals with high BMI and low insulin resistance (Borrell and Papapanou, 2005). Moreover, Al-Zahrani and co-workers reported a significant association between high BMI, waist-to-hip ratio, and periodontal disease in young adults (Al-Zahrani et al., 2003).

Osteopenia /Osteoporosis

Some studies reported that women with low bone mineral density have more clinical attachment loss, gingival recession, and gingival inflammation (Mohammad et al., 1997; Tezal et al., 2000; Inagaki and Noguchi, 2002). According to these studies, it has been hypothesized that low bone density in osteoporosis in combination with abnormal hormone function and genetic factors may affect the host immune system and increase susceptibility to periodontal diseases (Wactawski-Wende, 2001).

HIV disease

Patients with HIV have shown higher prevalence and severity of periodontitis (Winkler and Murray, 1987). However, in some studies, the progression of periodontal disease was not different between HIV-positive and HIV-negative groups (Robinson et al., 2000; Hofer et al., 2002). This might be

explained by the fact that some HIV-positive individuals might be under good control, with their immune system being virtually unchanged compared to healthy individuals.

Psychosocial factors

The mechanism of the potential impact of psychosocial factors in periodontal health is complex and not well understood. It has been suggested that psychosocial stress results in a change of behavior, i.e. smoking or poor oral hygiene (Genco et al., 1999), which in turn may lead to periodontitis. In a study of 1,426 subjects, it was reported that under a similar situation of financial stress, adults with poor coping behavior had more susceptibility to severe periodontitis than subjects with good coping strategies (Genco et al., 1999).

1.1.6: Diagnosis

A complete periodontal examination plays an important role in the detection and treatment of periodontal disease. This examination usually includes demographics, medical and dental history, radiographic findings, and clinical observations. The first step in detection of gingivitis is the observation of the shape, colour, and texture of gingival tissues. Common clinical signs of gingivitis include redness, swelling, and bleeding on probing (Armitage, 2004b). Redness and swelling are often seen together on the gingival margin. Bleeding on gentle probing results from minute blood ulceration and fragility of inflamed periodontal tissues and blood vessels (Armitage, 2004a). Specific periodontal measures include recording of pocket depth, clinical attachment level (Ramfjord, 1967), and plaque index (O'Leary et al., 1972) and are often taken at four to six sites around every tooth. Pocket depth is the distance between the tip of the gingival margin to the base of gingival crevice. The clinical attachment level and radiographic crestal bone levels are known to be indicators of past disease activity and damage caused by periodontitis. Clinical attachment level is the distance from the cement-enamel junction (CEJ) to the base of crevice measured with a periodontal probe (Ramfjord, 1967). Although using fewer sites in epidemiology surveys can be useful to estimate disease severity, it tends to underestimate prevalence of the disease (Ainamo et al., 1982; Stoltenberg et al., 1993).

1.1.7: Prevention and Treatment

Prevention of periodontal disease is based on the control of risk factors (Pihlstrom, 2001). When oral hygiene procedures are withdrawn, the biofilm begins to form a layer on the tooth in a 24-hour period and can result in gingivitis within 10 to 12 days (Loe et al., 1965). Tooth cleaning can reverse gingivitis into a healthy condition within one week (Loe et al., 1965; Sambunjak et al., 2011). Some studies have demonstrated that good oral hygiene, tooth brushing, flossing, and regular dental cleaning can prevent periodontal diseases and inhibit further clinical attachment loss (Loos et al., 1988; Brothwell et al., 1998; Berchier et al., 2008). A clinical study has found that using a toothbrush twice a day could reduce gingival bleeding by 35%, and when combined with flossing techniques at home resulted in an overall 67% reduction (Ahovuo-Saloranta et al., 2004). In addition, daily supervised tooth brushing in child populations can prevent gingivitis (Honkala et al., 1986). Importantly, tooth brushing and flossing have more effect on gingivitis than tooth brushing alone (Sambunjak et al., 2011).

Some publications have recommended for some patients to use chemotherapeutic agents daily as oral hygiene adjuncts in order to increase the efficacy of their oral hygiene methods. An oral rinse containing chlorhexidine 0.12%, an anti-bacterial agent, and fluoride-containing mouthrinses have been found to be effective to reduce supra-gingival plaque and gingivitis (Westfelt et al., 1983).

Initial therapy usually involves removing the supra- and subgingival biofilm and calculus around all accessible tooth surfaces through scaling and root planing (Armitage, 2004a). This procedure is efficient at reducing gingival inflammation and at preventing establishment and progression of periodontal diseases (Pihlstrom et al., 2005). However, most pathogens are not removed completely and remain in periodontal tissues and on the tooth surface due to anatomical structures, poor host defense, or bacterial invasion of soft tissues. Systemic antibiotic therapy can help the host defense system to control and remove the infection in certain refractory cases. However, using antibiotics has some disadvantages such as adverse drug reactions and increasing microorganism drug-resistance (Slots and Rams, 1990; Slots and Ting, 2002; Seiler and Herold, 2005). When patients do not respond to initial therapy, surgical therapy can also be effective in treating chronic periodontal disease (Heitz-Mayfield et al., 2002). Periodic periodontal maintenance must be part of a comprehensive therapeutic approach in order to maintain stability of the periodontium.

1.2: Dental Caries

1.2.1: Definition and Epidemiology

Dental caries is a result of imbalance of the indigenous bacteria that accumulate on the tooth surface, and occurs when demineralization of the hard tissue and destruction of the organic matter of the tooth is initiated by acid production of cariogenic bacteria (Kutsch and Young, 2011).

It is difficult to estimate the global prevalence and distribution of dental caries since the diagnosis criteria differ among studies (Selwitz et al., 2007). Despite the decrease in severity and prevalence of dental caries in developed countries, it is still one of the most common diseases especially in adults aged 35 and over (Brodeur et al., 2000). The 2007-2009 Canadian Health Measures Survey studied 5,586 Canadian people and reported that 96% of adults had one or more decayed, missing, or filled teeth (Health Canada, 2010). However, dentate adults had fewer teeth with untreated decay but had more teeth extracted and teeth filled. Also, Canadian males had more untreated tooth caries than females. Moreover, individuals with low income level have a higher amount of dental caries compared with people with higher income (Touger-Decker and van Loveren, 2003; Beltran-Aguilar et al., 2005; Health Canada, 2010). The National Health and Nutrition Examination Surveys (NHANES III) conducted in 1988–1994 and 1999–2004 in the United States reported tooth decay in 91% of dentate adults aged ≥ 20 years, 86.8% of dentate persons aged 20–39 years, 95.1% of people aged 40–59 years, and 93.1% of individuals aged more than 60 years (Beltran-Aguilar et al., 2005). In addition, dentate non-Hispanic white adults aged ≥ 20 years had higher coronal caries than non-Hispanic Black and Mexican-American individuals. Moreover, Kidd et al. found that English elderly individuals who live in their own homes had less root caries than those living in nursing homes (Kidd et al., 2000).

It was reported that generally women have demonstrated a higher level of dental caries than men (Lukacs, 2010). Three factors are related to the higher rate of caries among women (Lund, 2009):

- Female sex hormones which promote cavity formation.

- Flow rate of saliva in women is less than in men and results in reducing the removal of food debris from teeth.

- Food craving and alteration of immune response during pregnancy.

1.2.2: Stages of Disease

In 2002, the International Consensus Workshop on Dental Caries Clinical Trials developed a caries classification system divided into six clinical stages (Pitts, 2004; Shivakumar et al., 2009). Diagnostic threshold is a term that explains the cut-off level used to describe which lesions are "caries" and which are classified as "sound" (Pitts, 2004). This can be shown in the form of an "iceberg" of disease progression in which only the tip represents clinically detectable lesions (Figure 1.1).

The first stage is a sub-clinical lesion in a dynamic state of demineralization and remineralization with initial colonization of bacteria on the teeth. In the second stage, when the demineralization predominates, enamel progressively breaks down, a process that can be detected only with fiber-optic transillumination or bitewing radiographs. It has been many years since non-cavitated (or pre-cavitated) enamel lesions are detected and measured (Marthaler, 1984; Neilson and Pitts, 1991; Ismail, 1997; Pitts, 1997b; a; 2001). In the third stage, direct visualization after drying can detect non-cavitated enamel lesions clinically (white and brown spots) (D_1). Sound enamel is translucent and microporous. After repeated demineralization challenges, the first sign of caries is a change in translucency and light reflection after drying for a short time. Ekstrand et al. reported the histological depth of caries according to their severity (Ekstrand et al., 1995). They indicated that white spots with air-drying are limited to the outer half of the enamel. However, the depth of white spot lesions that are obvious without air-drying is located between the inner half of the enamel and the outer third of the dentin. In the fourth stage, acid starts to weaken and dissolve parts of the enamel and sub-surface enamel is dissolved away. The surface collapses and a cavity appears that can be detected clinically with an explorer or direct visualization and is limited to the enamel (D_2). When the enamel breaks down without visible dentin, caries extend to the middle third of the dentin and in case of cavities with visible dentin, decay extends to the inner third of the dentin (Ekstrand et al., 1995). In the fifth stage, caries extend to the underlying dentin in the absence of therapy (D_3). In the sixth and final stage, the bacteria can invade and infect the pulp tissue of the tooth and result into a large cavity (D_4).

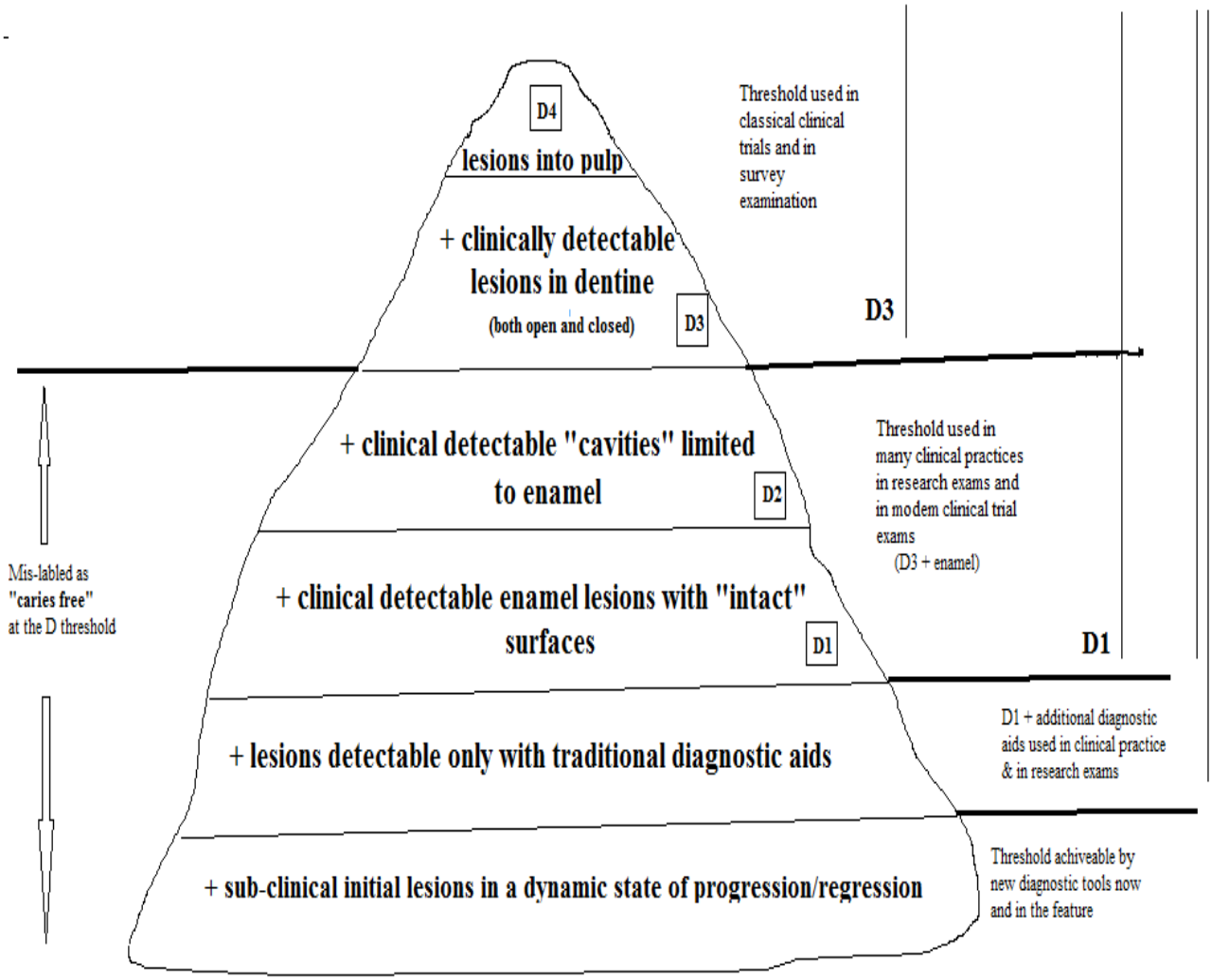

Figure 1.1: **Conceptualizing the Caries Process (Pitts, 2001)**

1.2.3: Etiology

Nowadays, most experts in cariology agree that caries is an infection that can be transmitted from one person to another and that its initiation and progression are influenced by several factors (Clarkson, 1999). Initially, it was proposed that the three main etiological factors for dental caries were the host,

including the hard tooth surfaces and saliva, carbohydrate substrates, and bacteria such as *mutans streptococci* and *lactobacilli* species (Keyes, 1960). When these three factors were present in the oral cavity, dental caries could occur. It was later found that the frequency with which teeth are exposed to the cariogenic environment influences caries development (Newbrun, 1989). Therefore, time became the fourth primary etiological factor that was added to the initial three essential factors (Figure 1.2). Preventive methods such as topical fluoride applications, oral hygiene instructions, and dietary counseling were then developed and showed clinical efficacy against caries. Consequently, Fejerskov and Manji added more factors implicated in the caries process to create a more comprehensive risk assessment model (Figure 1.3) (Fejerskov and Kidd, 2008).

The oral cavity contains a vast array of bacteria but only a few of them are believed to be cariogenic (Teanpaisan et al., 2007). *Lactobacilli* and *mutans streptococci* species have a mutual synergy and are positively correlated with dental caries (Loesche, 1986; Berkowitz, 2003; Beighton, 2005). Two strains of *mutans streptococci*, *S. mutans* and *S. sobrinus*, play an important role in the etiology of dental caries (Bowden, 1990). Many studies have demonstrated the role of *S. mutans* in the initiation of dental caries while *lactobacilli* are known to play a role in the progression of carious lesions (Tanzer et al., 2001; Nishikawara et al., 2006). Higher amounts of *S. mutans* are seen in enamel caries compared to root caries (Brown et al., 1986). A recent study found a high prevalence of *lactobacilli* in root caries in elderly people, confirming earlier results (Preza et al., 2009). Many theories have been postulated to explain dental caries. As early as 1890, Miller brought forward the "Acid-parasitic Theory" of dental caries. He described oral microorganisms as capable of initiating caries by producing acid from dietary carbohydrates. These acid products lead to the loss of the mineral composition of the tooth (Miller, 1890). The "Proteolytic Theory" was then brought forward by Gottlieb in 1944 (Gottlieb, 1944). He theorized that initiation of caries was due to proteolytic enzymes of bacteria targeting protein elements of the tooth, such as lamellae and rod sheaths, and destroying the matrix of enamel by dissolving the enamel apatite crystals. Decades later, Schatz and Martin reported the proteolysis chelation mechanism (Schatz and Martin, 1962). This theory supported the simultaneous microbial degradation of the organic components known as proteolysis, and the remineralization of the tooth by the process known as chelation. The current etiologic concept of dental caries focusing on the effect of cariogenic bacteria-producing organic acid in fermentation of carbohydrates was first discovered and developed in the 1970s (Loesche, 1976). The main organic acids in the presence of fermentable carbohydrates are lactic, formic, and acetic acids. These acids contribute to decrease the pH of the oral biofilm located on the

tooth surface, causing surface demineralization and providing an advantageous environment for *Streptococcus mutans*. These bacteria are responsible for the cariogenicity of the dental plaque by expressing wide range of virulence factors.

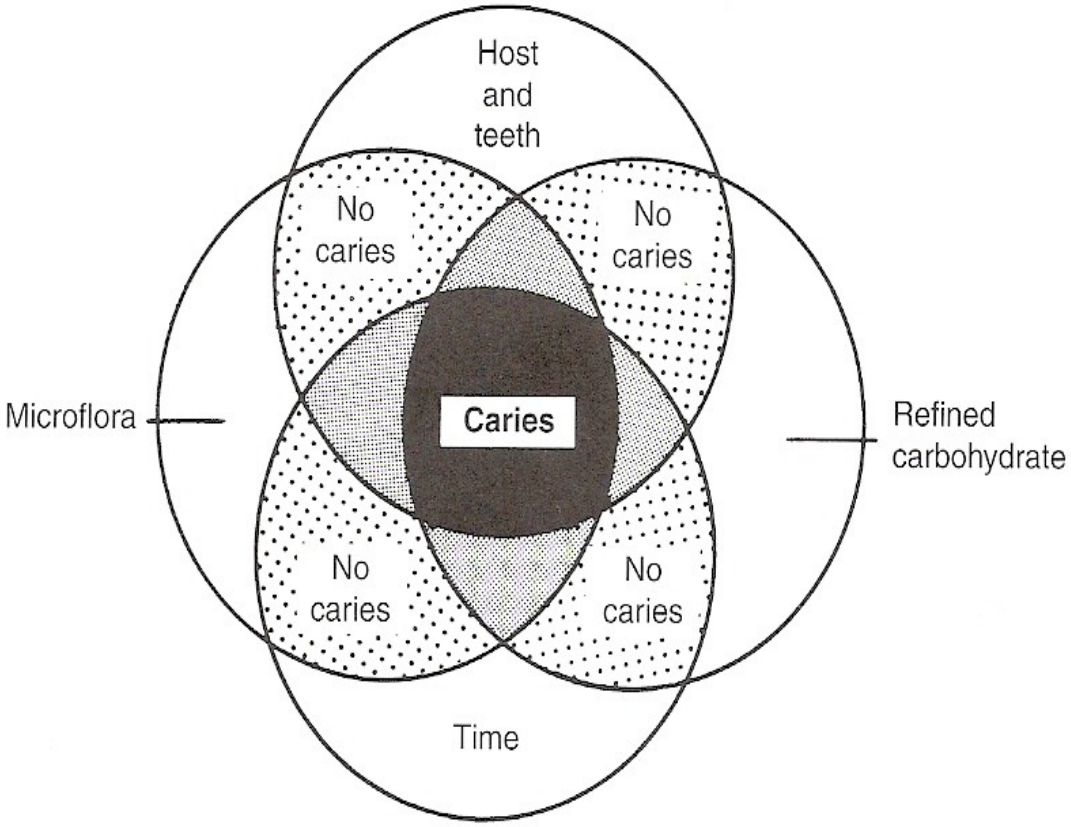

Figure 1.2: Main Etiological Factors of Dental Caries (Selwitz et al., 2007)

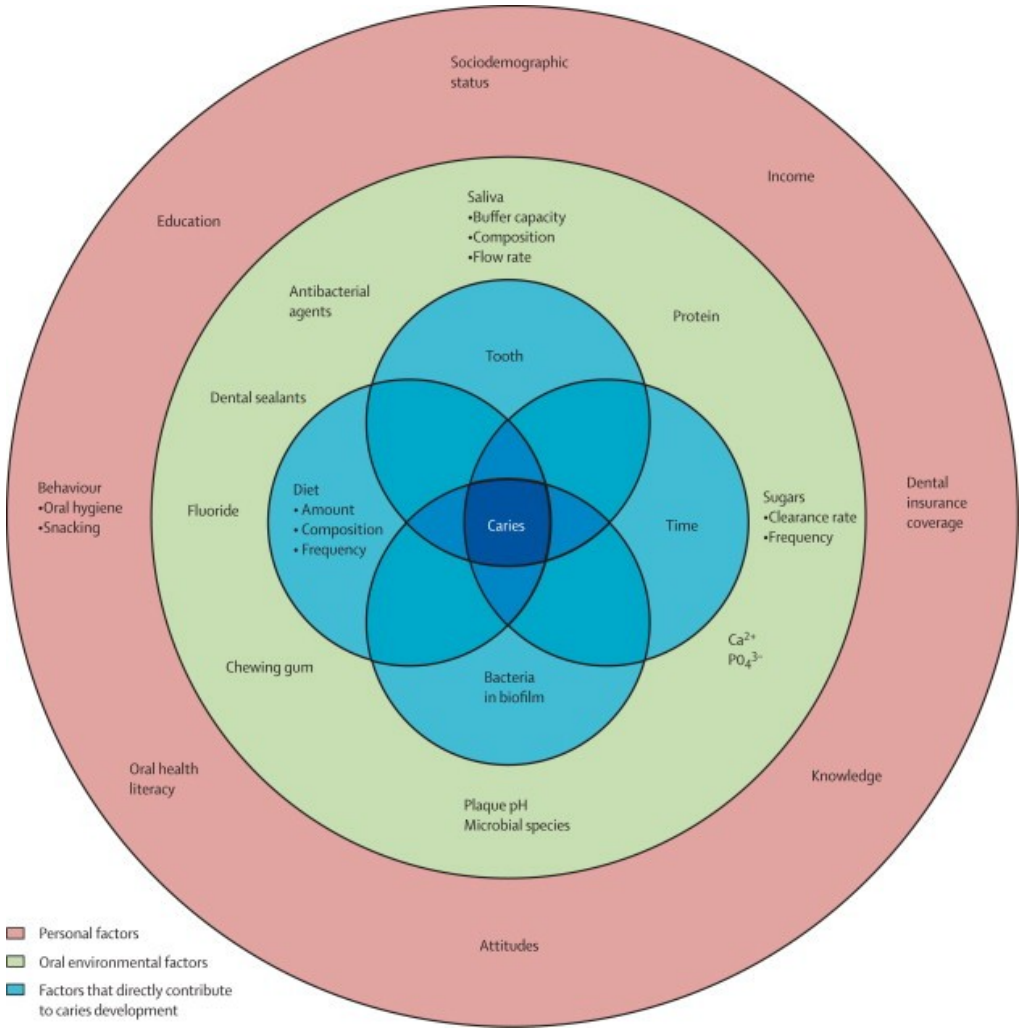

Figure 1.3: Risk Factors of Dental Caries (Fejerskov and Kidd, 2008)

1.2.4: Pathologic Pathways

The main pathogenic characteristics of *mutans streptococci* and *lactobacilli* are adhesion, acid production, and acid tolerance (Marsh, 2009). *S. mutans* is a facultative anaerobic bacterium producing lactic acid, surface antigen I/II, and a water-insoluble glucan (Hamada and Slade, 1980; Takahashi and Nyvad, 2011). Its adherence to the tooth surface is formed mainly by proteins such as glucane-binding that is triggered by the enzymatic actions of glucose transferases and water-insoluble glucans (WIGs) (Loesche, 1986; Simon, 2007). WIGs play an important role in developing initial caries through the

adhesion and aggregation processes (Mattos-Graner et al., 2000). This protein modifies physio-chemical properties of dental plaque and increases porosity of the dental plaque matrix in order to make it more cariogenic (Zero et al., 1992; Cury et al., 1997; Napimoga et al., 2004). Also, WIG decreases the calcium and phosphate concentrations in the saliva, decreasing the saliva buffer action (Napimoga et al., 2004). In addition, colonization of *mutans streptococci* on the tooth surface is mediated via both sucrose-independent and sucrose-dependent adhesion metabolisms. The attachment process is initiated by sucrose-independent adhesion to salivary components within the acquired enamel pellicle, while sucrose-dependent adhesion may be primarily responsible for establishing permanent colonization (Loesche, 1986). The surface protein P1, also known as antigen I/II (ca. 185 kDa), is the surface adhesion protein that is released by *S. mutans* and other streptococci and influences the sucrose-independent adhesion metabolism (Lee, 1992; Whittaker et al., 1996). Consequently, these microbial acid-induced adaptation and selection processes change the demineralization/mineralization balance and shift it toward mineral loss (Takahashi and Nyvad, 2011). On the other hand, *S. mutans* can resist other types of bacteria and thrive at low pH levels and in high glucose concentrations (Loesche, 1986). This can result in the maintenance of an acidic environment and demineralization of the tooth surface, resulting in caries.

When the lesion progresses to the stage of cavitation, the organisms penetrate into the enamel crystals and underlying dentine. In the advanced clinical stage of carious lesions, *mutans streptococci* do not have ability to survive at pH levels below 5.0, and *lactobacilli*, as a secondary cariogenic bacteria, can be found in higher concentrations (Loesche, 2007). *Lactobacillus* is a gram-positive facultative anaerobic bacteria which is rod-shaped and non-spore forming (Falsen et al., 1999). It derives all its energy from the metabolism of glucans into acid fermentation and can also tolerate a low pH environment (Marcotte and Lavoie, 1998). Specific mechanisms by which *lactobacilli* can adhere to the tooth surface are not well understood. Recognition of collagen, a major component of dentine, might be a mechanism used by these bacteria to adhere to root surfaces as well as caries (McGrady et al., 1995; Featherstone, 2000; Love and Jenkinson, 2002).

1.2.5: Risk Factors

Inadequate Salivary flow and composition

Saliva contributes to maintain balanced oral flora that promote health and integrity of tooth surfaces (Hicks et al., 2003; Garcia-Godoy and Hicks, 2008; Kutsch and Young, 2011). Saliva protects teeth against caries with mechanisms including bacterial clearance, direct antibacterial activity, buffering, and remineralization. The buffering capacity and the ability of saliva to lubricate the oral cavity and wash away microorganisms and food debris from the tissues and teeth, help in balancing the demineralization/remineralization process on the tooth surface (Dowd, 1999; Stookey, 2008; Hara and Zero, 2010; Paice et al., 2011). For example, a reduction in the salivary flow causes an inadequate saliva buffering action, resulting in a decrease of the oral pH. Therefore, the host inability to counterbalance the acidic environment will create an ideal condition for the establishment of cariogenic bacteria (Simmonds et al., 2000). Moreover, saliva has a significant antibacterial activity through its enzymes that can contribute to tooth health (Spielmann and Wong, 2011). Some medications, medical conditions, and radiotherapy to the head and neck region can cause a reduction of saliva flow and dry mouth (xerostomia) resulting in more caries (Spielmann and Wong, 2011).

Oral levels of cariogenic bacteria

Tooth caries are caused by an imbalance of indigenous bacteria and can be transmitted as early as the infant stage (Caufield and Griffen, 2000; Harris et al., 2004). Many studies have demonstrated the role of *S. mutans* in initiating dental caries, while *lactobacilli* are known to have a role in the progression of carious lesions (Tanzer et al., 2001; Nishikawara et al., 2006). Teanpaisan et al. studied the acquisition of *mutans streptococci* and *lactobacilli* species in relation to dental caries. They used saliva of 3 to 24-month-old Thai children as a medium for colonization by *mutans streptococci* and *lactobacilli* (Teanpaisan et al., 2007). Subjects who had a significant early colonization by *mutans streptococci* and *lactobacilli* showed a higher number of dental caries than whose saliva exhibited later colonization. The authors found a significant association between high *mutans streptococci* and *lactobacilli* salivary levels and tooth caries. The level of cariogenic bacteria differs among individuals of different age

groups. For example, level of *lactobacilli* in saliva increases with age, particularly in individuals aged 70 years and older, while the prevalence of *mutans streptococci* in saliva or plaque is not related to age (Percival et al., 1991).

Diet

Sugar is a major component of our daily diet. It has a fundamental role in caries development since it is used directly by cariogenic bacteria (Mobley et al., 2009). Sugar and fermentable carbohydrates are metabolized into acids by cariogenic bacteria, resulting in low pH of the oral environment and enhancing growth of acidogenic and aciduric bacteria such as *mutans streptococci*. The initial colonization of *mutans streptococci* on the tooth surface is facilitated by sucrose. In contrast, a diet with lower intake of sugar and more calcium-rich nutrients can favor remineralization and prevent caries (Touger-Decker and van Loveren, 2003; Makinen, 2010). However, the frequency of fermentable carbohydrate intake seems to be correlated to caries more importantly than the total amount of sugar in the diet (Paes Leme et al., 2006). The 1945–1953 Vipeholm study is one of the largest clinical studies investigating the relationship between the amount of sugar intake and frequency of dental caries (Gustafsson et al., 1954). The authors concluded that consumption of sugar during meals and between meals increases the risk of dental caries. In summary, dietary factors that are related to the incidence of dental caries are:

- Amount of fermentable carbohydrate consumption;
- Concentration of sugar in food;
- The duration teeth are exposed to carbohydrates;
- Frequency of meals and snacks;
- Sequence of food consumption.

Poor Oral hygiene

On the basis of existing literature, oral hygiene status appears to be an important factor in caries incidence (Andlaw, 1978; Bellini et al., 1981; Selwitz et al., 2007). The European Workshop on Mechanical Plaque Control approved the following theory in 1998: “Forty years of experimental research, clinical trials and demonstration projects in different geographical and social settings have

confirmed that effective removal of dental plaque is essential to dental and periodontal health throughout life" (Lang et al., 1998).

The use of fluoride toothpaste and toothbrush is the most common form of oral hygiene practiced by individuals (Selwitz et al., 2007). Mechanical oral hygiene measures such as tooth brushing and dental floss can remove the oral biofilm and food debris from the smooth and interdental surfaces of teeth (Fejerskov and Kidd, 2008). Also, toothpaste provides an ideal vehicle for the carriage of agents like fluoride for the prevention of dental caries. Papas and coworkers demonstrated that the toothbrush had the beneficial effect of improving the oral hygiene of patients with medically induced xerostomia (Papas et al., 2007). Moreover, Bellini et al. indicated that the quality of tooth cleaning was more effective than its frequency (Bellini et al., 1981).

Fluoride Exposure

Dental caries have decreased in prevalence in developed countries over the years. This decline can be explained in part due to the widespread use of fluoride, both topically and in public water supplies (Buzalaf et al., 2011). Therefore, insufficient systemic or topical fluoride exposure can enhance the prevalence of dental caries (Selwitz et al., 2007).

Smoking

Tobacco use, notably cigarette and pipe smoking, is considered a risk factor for dental caries (Hirsch et al., 1991; Jette et al., 1993; Axelsson et al., 1998). It causes bad breath, tooth discoloration, accumulation of plaque and tartar, and compromises salivary flow (Lie et al., 2001; Rooban et al., 2011). The oral levels of *lactobacilli* and *Streptococcus mutans* were shown to increase in smokers, thereby explaining the association between smoking and dental caries (Sakki and Knuuttila, 1996).

Social factors

Dental caries are significantly related to low income, low level of education, and lack of dental insurance coverage (Burt et al., 1999). Therefore, dental health education through school systems and

oral health professionals, as well as an increase in public awareness of oral health through advertisements, could explain the decrease in the prevalence of dental caries worldwide.

Medications, systemic diseases, and physical/mental disabilities

Some medications and systemic diseases can lead to xerostomia and ultimately, dental caries due to the reduced buffering action and quantity of saliva. For example, medications such as antihistamines and antidepressants, patients receiving radiotherapy to the head and neck, and some medical conditions like diabetes mellitus, diabetes insipidus, Parkinson's disease, and sarcoidosis as well as autoimmune diseases like HIV/AIDS and Sjogren's syndrome can cause xerostomia (Ship, 2003; Scully and Felix, 2005; Kielbassa et al., 2006; Neville, 2007; Seo et al., 2009; Johnson, 2010). In addition, a wide variety of physical and mental disabilities can result in a reduced frequency and quality of oral hygiene measures, exposing individuals to caries.

Local and iatrogenic factors

Presence of gingival recessions exposing root surfaces that are less resistant to caries (Selwitz et al., 2007), use of orthodontic appliances (Ogaard et al., 1988; van der Veen et al., 2010), poorly designed or ill-fitting fixed or removable prostheses (Featherstone et al., 2011), and defectuous restorations (Kidd et al., 2000; Aleksejūnienė et al., 2009) can result in an increase in the amount of dental caries.

1.2.6: Diagnosis

The detection and classification of dental caries is a complex task in clinical research (Ricketts et al., 1997). Investigators have studied the use of several procedures to detect dental caries (Stookey and Gonzalez-Cabezas, 2001). Different technologies are used depending on the severity, depth, and location of the carious lesion. The subclinical carious lesion in a dynamic state of demineralization and remineralization (first stage) can be detected with quantitative light-induced fluorescence (QLF) technology, near-infrared fluorescence (DIAGNOdent®, KaVo Dental GmbH, Biberach, Germany), electronic caries monitor, and digital radiography. QLF is a diagnostic tool for assessment of dental

caries lesions, dental plaque, and bacterial activity. It is also used to detect parameters like percentage of mineralization lost as well as lesion depth and size. However, it seems to be limited to a lesion depth of about 400 microns and cannot detect occlusal or secondary caries (Amaechi and Higham, 2002; Heinrich-Weltzien et al., 2005). DIAGNOdent® is an examination tool for detecting early and very small caries on occlusal surfaces (Kuhnisch et al., 2007). However, the instrument's sensivity can be reduced by factors like presence of plaque, staining and dehydration of teeth (Lussi et al., 1999). Both methods have the same merit in quantification of smooth surface caries, although research shows that QLF can analyze changes in mineral content with better precision (Shi et al., 2001). Non-cavitated carious lesions associated with enamel breakdown (second stage) are detected only by fiber-optic transillumination technology or bitewing radiographs. Proximal lesions were diagnosed by fiber-optic transillumination two times more than was the case for clinical examination. Digital fiber-optic transillumination can diagnose early caries and even lesions that cannot be detected with radiography by using safe white light or standard light. In proximal surfaces, light is shone from one surface and captured from the opposite surface by the camera. In occlusal surfaces, the mouthpiece illuminates the tooth from both facial and lingual surfaces and images from the top of the tooth. The images are sent to the computer and analyzed with algorithms (Schneiderman et al., 1997). White carious lesions (third stage) with appearance of sound surfaces are visually detected only when forced air is blown on the suspected surfaces. The aim is to eliminate moisture and change the properties of the unmineralized enamel surface. A dental explorer is used for the detection of cavitated dental caries (fourth stage) involving the enamel of the occlusal, facial, and lingual surfaces.

In 1968, Radike reported clinical criteria for detection of frank carious lesions (Ismail, 2004). These criteria rely on visual detection, the use of gentle exploring with a dull-ended explorer for detection of caries, or radiographic images. Frank lesions are usually easily detected and the cavity corresponds to either a discontinuity of the enamel surface caused by loss of tooth surface or a cavitation resulting from the caries process. However, lesions that are not cavitated are more difficult to detect. These non-cavitated lesions are considered caries if the explorer "catches" or resists upon removal of the explorer after its insertion into a pit or fissure with a moderate to firm pressure or if a surface loses the translucency of its enamel adjacent to a pit compared to the surrounding tooth structure. They are also considered caries if the surfaces are etched or if there are white spots showing subsurface demineralization when an explorer or blown air is used. For detection of interproximal caries, the procedure for detection includes a combination of visual-tactile methods, radiographs, and

transillumination. A bitewing radiograph is recommended to detect any break in continuity of enamel surfaces caused by caries. Transillumination is mostly used for anterior teeth and will reveal a loss of translucency on calculus-free and stain-free proximal surfaces when caries are present. Root caries are mostly seen in the maxillary arch and on facial surfaces of exposed roots of mandibular teeth and can be easily detected most of the time with a visual and tactile examination (Katz et al., 1982). They are encountered in the presence of gingival recessions that leave the roots exposed to the cariogenic flora. NIDCR criteria for root caries are lesions in root surfaces that are yellow/orange, tan, or light brown (National Institute of Dental Research (U.S.). Epidemiology and Oral Disease Prevention Program., 1987). These lesions show softness to an explorer tip.

1.2.7: Prevention and Treatment

Daily removal of plaque by tooth brushing, fluoride toothpaste and dental flossing are among the most efficient ways to remove plaque and prevent caries on the tooth surfaces (Hamilton, 1977). Flossing helps in removing the debris and oral biofilm from the interproximal surfaces (Bellini et al., 1981; Roberson et al., 2006; Selwitz et al., 2007). Dietary changes such as substitution of sugar-free foods for snacks are effective at diminishing caries prevalence (Touger-Decker and van Loveren, 2003; Makinen, 2010). Reducing sugar consumption and careful oral hygiene can therefore reduce the incidence of dental caries. Another preventive method against caries is the use of pit and fissure sealants in children and adolescents aged less than 20 years (Simonsen and Neal, 2011). It aims at preventing the colonization of cariogenic bacteria into pits and fissures, areas that are hard to reach with daily oral hygiene measures, with a durable restorative material.

There are many caries prevention programs like fluoridation of water, salt, mouthrinses, and intake of fluoride tablets in schools (Selwitz et al., 2007). Fluoride can bind to hydroxyapatite crystals of the enamel (Robinson, 2009; Denbesten et al., 2011) and increase the resistance of tooth structure to demineralization. Therefore, it has a significant protective effect against bacterial carbohydrate metabolism and even reduces bacterial-driven acid formation (Balzar Ekenback et al., 2001). Fluoride can stimulate remineralization in small carious lesions but in deeper ones, the caries will have to be removed and restored to prevent the progression of caries into the tooth structure. It is generally recommended to provide topical fluoride like fluoride varnishes and gels to high-risk populations like

infants and patients with reduced salivary gland functions in a professional setting (Roberson et al., 2006; Tubert-Jeannin et al., 2011). Varnishes and gels provide high uptake of fluoride ion into enamel and have shown clinical efficacy (Lee et al., 2010).

The individual risk factors and the extent of caries dictate the treatment regimen. For small lesions, topical fluoride is often effective at promoting tooth remineralization and treating caries. However, in cavitated advanced lesions, the missing tooth structure is replaced by a restoration. For many years, dentists have use mechanical removal and tooth reconstruction with restorative materials to prevent and treat caries, and to restore the tooth structure, function, and aesthetic. When caries are too extensive and the pulp of the tooth is infected, a root canal therapy may be advised to prevent further infections. If there is not enough tooth structure remaining, replacing the tooth structure with a restoration may not be possible and dental extraction might be the only option (Roberson et al., 2006). Periodic recalls based on individual risk factors and including a dental examination, radiographs, and oral prophylaxis are recommended to monitor the patient's caries status and detect early carious lesions. Therefore, a caries management program combining restorative therapy with a preventive approach and a comprehensive maintenance phase will reduce caries incidence.

1.3: Current Knowledge on the Association between Caries and Periodontal Diseases

1.3.1: *In Vitro* Studies

The potential association between periodontal pathogens and cariogenic bacteria has been studied using different *in vitro* models. Four strains of periodontopathogens, *F. nucleatum (F. n.), A. actinomycetemcomitans (A. a.), P. gingivalis (P. g.), Prevotella intermedia (P. i.)* and four strains of cariogenic bacteria*, S. mutans (S. m.), S. sanguis (S. s.), A. viscosus (A. v.),* and *L. acidophilus (L. a.)* were used in an *in vitro* study (Rao and De-Yi, 2005). The authors found that the co-aggregations of periodontopathogens and cariogenic bacteria were specific. Fine and colleagues studied the relationship between *A. actinomycetemcomitans* and cariogenic bacteria growth in an *in vitro* model using an agar diffusion method. The agar diffusion assay showed that *A. actinomycetemcomitans* had an inhibitory effect on cariogenic bacteria. The authors found that the saliva from *A. actinomycetemcomitans*-positive subjects killed *S. mutans*. (Fine et al., 2007). Another *in vitro* study of the effect of oral streptococci on anaerobic bacteria such as *P. intermedia, F. nucleatum, Veillonella, P. gingivalis,* and *P. micros* showed that these anaerobic bacteria were inhibited by certain strains of streptococci, particularly *S. mutans* and *S. salivarius*, as a result of their lactic acid production (Doran et al., 2004). Alakomi et al. observed that lactic acid produced by *lactobacilli* can permeate the outer membrane of gram-negative bacteria and induce the inactivation of periodontopathogens (Alakomi et al., 2000). These studies suggest that there are interactions between cariogenic bacteria and periodontal pathogens.

1.3.2: Human Studies

Findings of human studies on the association between caries and periodontal diseases are controversial. Several clinical studies did not find any relationship, either positive or negative, between dental caries and periodontal diseases (Miller and Seidler, 1940; Brucker, 1943; Massler et al., 1952; Kinane et al.,

1991). The authors have suggested that this is due to the fact that these two diseases have different risk factors. Miller and Seidler studied the potential association between the two common oral diseases among teenagers aged 14 to 18 years and did not find any correlation (Miller and Seidler, 1940). In 1952, Massler et al. computed the average DMF score among three different groups of subjects with no gingivitis, moderate, and severe gingivitis (Massler et al., 1952). The study included 4,043 white males aged between 17 and 20 years old from various areas of the United States. No association was found between caries and gingivitis. In 1991, Kinane et al. studied 800 patients by using their radiographs. The level of bone loss was measured for assessment of periodontal disease. The number of decayed and filled teeth (DFT) was recorded. The relationship between caries and periodontal disease was analyzed and the data was stratified for different categories of sex, age, and the number of teeth present. The results indicated there was no association between these two oral diseases (Kinane et al., 1991).

On the other hand, more recent clinical studies have found a positive association between caries and periodontal diseases (Vehkalahti and Paunio, 1994; Albandar et al., 1996; Ekstrand et al., 1998; Saotome et al., 2006). In a clinical study of 368 elderly individuals, Saotome et al. investigated the relationship between cariogenic bacteria salivary levels, the periodontal status, and root surface caries (Saotome et al., 2006). The investigators found that attachment loss increased the probability of having high oral levels of *lactobacilli*, thus increasing the risk of root surface caries. They concluded that the measurements of *lactobacilli* and *mutans streptococci* could be useful indicators of root caries in elderly individuals with attachment loss. In addition, another study found an association between root caries and clinical signs of periodontal disease such as deep pocket depth in Finnish males (Vehkalahti and Paunio, 1994). In younger subjects, the results of a study showed that the presence of bleeding upon probing was related to cases with progressing caries (Ekstrand et al., 1998). Moreover, Albandar et al. reported that patients with aggressive periodontitis exhibited high prevalence of dental caries (Albandar et al., 1996).

Some studies have reported a negative association between caries and periodontal diseases (Fine et al., 1984; Sioson et al., 2000; Iwano et al., 2010). Fine et al. found that a low number of proximal carious lesions were found in the presence of juvenile periodontitis compared to age- and sex-matched individuals (Fine et al., 1984). A matched cross-sectional clinical study found that juvenile periodontitis patients had indeed significantly less proximal caries than their matched controls without periodontitis (Sioson et al., 2000). A more recent study looked at the potential microbiological

correlation between caries and periodontal diseases in Japanese adults (Iwano et al., 2010). The investigators studied salivary levels of *S. mutans* and *P. gingivalis* in relation to the periodontal and caries status. Forty subjects aged from 23 to 78 years participated in the study. Periodontal disease was defined as having a mean pocket depth ≥ 3mm. Subjects were divided in three groups: those having caries and no periodontitis, those having periodontitis and caries, and those having periodontitis and no caries. No subject had a healthy periodontium and no caries. All subjects had a clinical examination including plaque and bleeding indices, pocket depth, and number of decayed surfaces. Salivary levels of *S. mutans* and *P. gingivalis* were evaluated by using real-time PCR methods. Thereafter, 10 periodontitis patients had scaling and root planing, and two to four months later provided salivary samples to detect *S. mutans* and *P. gingivalis*. The authors found that the concentration of *P. gingivalis* significantly decreased after treatment while *S. mutans* levels increased. Also, clinical parameters of periodontal disease were significantly improved after therapy. Therefore, they concluded that there was an inverse relationship between caries and periodontal disease according to clinical and microbiological findings.

CHAPTER TWO

Material and Methods

2: Material and Methods

2.1: Problem Statement

Very little is known about the risk of caries in patients with periodontal diseases and conflicting results are reported based on different clinical studies. This study is among the first investigations to assess the risk of caries in adults suffering from chronic periodontitis, and to compare their levels of periodontal and caries pathogens with those with healthy periodontium.

2.2: Hypotheses

The primary hypothesis of this study was:

There are no differences between individuals with a healthy periodontium and those with chronic periodontitis in terms of caries' risks and caries' clinical and microbiological measures.

The secondary hypothesis was:

Within subjects, oral levels of periodontal pathogens are not associated with oral levels of cariogenic bacteria.

2.3: Research Aims

The main objective of this study was:

> To assess if adults with chronic periodontitis were more prone to caries than those with a healthy periodontium.

The secondary objective was:

> To compare the oral levels of the major microbial pathogens of periodontal diseases such as *F. nucleatum, P. gingivalis, P. intermedia, T. denticola, T. forsythia, A. actinomycetemcomitans* and the levels of cariogenic bacteria such as *S. mutans* between adults with a healthy periodontium and those with chronic periodontitis, and to examine if there is any association between the level of periodontal pathogens and the levels of *S. mutans* in a cross-sectional analysis.

2.4: Study Design and Study Population

In this cross-sectional clinical study, patients with chronic periodontitis and healthy periodontium were recruited from the Université de Montreal and McGill University dental clinics, and through newspaper advertisement.

Subjects were eligible to participate to the study if there were at least 30 years old. Subjects were excluded if they had one of the conditions presented in Table 2.1. The Université de Montréal ethics committee approved the protocol for this study and informed written consent was obtained from each participant (Appendices I and II).

The study population was composed of 38 subjects with chronic periodontitis, and 58 subjects with a healthy periodontium. Chronic periodontitis was defined as having at least 4 teeth with ≥ 1 site with a pocket depth ≥ 4 mm and a clinical attachment loss ≥ 2 mm. Each group was then divided into two

subclasses (with and without untreated caries) according to their caries status as defined by Radike and NIDCR criteria (Radike, 1972; National Institute of Dental Research (U.S.). Epidemiology and Oral Disease Prevention Program., 1987)

Table 2.1. Exclusion criteria

Having less than 20 natural teeth
Any contraindication to dental examination due to a medical condition
Taking antibiotics 3 months prior study procedures or antibioprophylaxis needed prior dental examination
Having a dental cleaning 3 months prior study procedures
Diagnosis of aggressive periodontitis
Untreated acute oral infections
Unable to handle study procedures
Unable to provide consent

2.5: Data collection:

2.5.1: Medical and Socio-demographic Questionnaires

All participants were interviewed and medical and socio-demographic questionnaires were filled out by a trained dentist (Appendices III and IV).

2.5.2: Clinical Examination

A calibration trial was performed on 10 volunteer patients who were attending the undergraduate clinics at the Faculty of Dentistry of the Université de Montréal to calibrate the examiner (general dentist) under the supervision of a periodontist and to measure intra-examiner reliability for caries and periodontal measures. Full mouth (except third molars) coronal and root caries evaluation was conducted to score the number of decayed, missing, and filled teeth and teeth surfaces (DMFT/DMF_3S indices, Appendix V) (Broadbent and Thomson, 2005). The diagnosis of coronal and root caries was carried out based on criteria described by Radike as published in the Proceeding of the Conference on Clinical Testing of Cariostatic Agents (Radike, 1972) and NIDCR (National Institute of Dental Research (U.S.). Epidemiology and Oral Disease Prevention Program., 1987). Non-vital teeth were scored in the same manner as vital teeth.

Periodontal measures were performed with a manual periodontal probe (UNC-15, Hu-Friedy Mfg Co., Chicago, USA). Measurements were performed at mesio-buccal, buccal, and disto-lingual aspects of teeth in two randomly selected quadrants (Appendix VI). Linear measurements were rounded down to the lowest millimeter. Measurements included probing depth (PD), distance between the cemento-enamel junction and marginal edge of the free gingiva (GM-CEJ), bleeding index (BI) to evaluate gingival inflammation (Carter and Barnes, 1974), and plaque index (PI) to assess the oral hygiene status (O'Leary et al., 1972). The clinical attachment level was calculated by software (Filemaker Pro®, Filemaker Inc., Santa Clara, USA). The calculation was done by subtracting the GM-CEJ distance from the pocket depth (Ramfjord, 1967).

2.5.3: Microbiological Investigation

Bacterial detection by PCR

Supragingival and subgingival plaque samples were collected for each patient in the four quadrants of the mouth with a sterile curette. Plaque samples were placed in a tube containing 500 µl of neutral phosphate buffered saline (PBS, 10 mM, pH 7.4). Supragingival plaque samples were collected from four supragingival restoration-free and caries-free tooth surfaces and subgingival samples were taken from eight deep periodontal pockets (PD ≥ 5 mm) in the four quadrants. Thereafter, samples were immediately identified and stored in a freezer at -80°C for future microbiological analyses.

Seven microorganisms were selected for evaluation of supra- and subgingival plaques by polymerase chain reaction (PCR) techniques. These included parodontal pathogens such as *F. nucleatum, P. gingivalis, P. intermedia, T. denticola, T. forsythia,* and *A. actinomycetemcomitans* for chronic periodontitis (Nishihara and Koseki, 2004) and cariogenic bacteria such as *S. mutans* (Takahashi and Nyvad, 2011). Pure cultures of each bacterial species were used as positive controls. Negative controls were sterile DNase-free and RNase-free distilled water. Sequences of the PCR primers used for the targeted bacterial species are listed with their respective references in Table 2.2. The PCR amplification procedures were performed as described in the related articles for each bacterial species. Briefly, PCR reagents for all the targeted bacteria included a PCR buffer containing 200 mM of Tris-HCL (pH = 8.4), 500 mM of KCl, and $MgCl_2$ (Invitrogen™, Life Technologies Co., Carlsbad, USA), and a PCR buffer containing $MgCl_2$ (10mM Tris-HCL, 50 mM KCL, 1.5 mM MgCl2, at pH of 8.3) to adjust concentration of $MgCl_2$ as needed. In addition, a standard Taq buffer (Taq PCR Kit™, New England Biolabs Inc., Ipswich, USA) and 10 mM dNTPs (Invitrogen™, Life Technologies Co.) were used. All PCR amplification procedures were performed in a thermal cycler (PE2400™; Life Technologies Co.).

PCR reaction mixture of 50 µl for *F. nucleatum* contained 0.25 µl of 5U of Taq DNA polymerase, 1.5 mM of $MgCl_2$, 0.2mM of each dNTP, and 0.5 µM concentration of each primer (Fouad et al., 2002). Amplification reaction for *P. intermedia* was performed in 25 µl, including 0.016 mM of dNTP, 1mM of $MgCl_2$, 0.0006 nM of each primer, and 0.05U of Taq (Garcia et al., 1998). For *T. denticola,* the total reaction volume was 50 µl PCR reaction mixture. It contained 0.004 mM of dNTP, 0.0005 nM of each primer, 1.25 U of Taq, and 0.75 mM of $MgCl_2$ (Ashimoto et al., 1996). The amplification reaction

mixture volume for *P. gingivalis* contained 25 µl of reaction mixture including 0.001 mM of each primer, 2.5 unit of Taq polymerase, and 0.004 mM of dNTP in one buffer containing 1.5 mM of MgCl2 (Tran and Rudney, 1999). *A. actinomycetemcomitans* PCR mixture had a volume of 53.6 µl including 2.9 mM of MgCl2, 0.15µM of primer AaF, 0.47 µM of primer C11R, 10 U of Taq, and the deoxynucleotide triphosphate included 200 µM of each of the following components: dATP, dCTP, dGTP, and dTTP (Tran and Rudney, 1999). The PCR reaction mixture of 25 µl for *T. forsythia* consisted of 10x buffer, 200 µM of dNTP, 1.2 µM of each primer, 2.5 unit of Taq in 1x buffer containing 1.5 mM of $MgCl_2$ (Guillot and Mouton, 1996). For detection of *S. mutans*, the PCR reaction mixture of 25 µl contained a 10x buffer, 2.5 mM of each dNTP, 10 pmol/µl primers, 5 units of Taq DNA polymerase, and 50 mM of $MgCl_2$ (Psoter et al., 2011). PCR conditions for each primer pairs were optimized in pilot experiments.

The PCR condition for *F. nucleatum* included the initial denaturation process at 95°C (5 min), followed by 35 cycles (95°C for 1 min, 60 °C for 1 min, 72°C for 2 min, 72°C for 10s). The PCR amplification conditions for *P. intermedia* were 94°C (5 min), followed by 35 cycles (95°C for 1 min, 68 °C for 45s, 72°C for 2 min and 30s, 72°C for 10 min). Mixtures of *T. denticola and P. gingivalis* were incubated with initial denaturation step at 94°C (2 min), and then 30 cycles (94°C for 15s, 60°C for 15s, 72°C for 45s, 72°C for 5 min). *A. actinomycetemcomitans* mixture was performed at 95°C for 10min, followed by 35 cycles (95°C for 5 min, 61°C for 1 min, 72°C for 5 min, 75°C for 10 min). PCR conditions for *S. mutans* were performed at 95°C (15 min), followed by 35 cycles at (95°C for 15s, 56°C for 30s, 72°C for 1 min, 72°C for 7 min). PCR conditions for *T. forsythia* were performed at 94°C for 5 min followed by 30 cycles (94°C for 1 min, 32°C for 1 min 30s, 72°C for 2 min, 72°C for 7 min).

All the PCR products were cooled to 4°C before being taken out of the thermal cycler. PCR amplified samples were analyzed on a 2.5% agarose gel electrophoresis run at 80 V for a 45-minute period. Gels were immersed in TAE electrophoresis buffer consisting of a mixture of 40mM Tris-Acetate and 1 mM of EDTA (pH 8.3). . Fifteen µl of PCR amplified material were loaded on each gel with 10x of Blue Juice™ Loading Buffer (Life Technologies Co.). The ladder used was a 100 bp DNA ladder (Life Technologies Co.). The gels were then stained with ethidium bromid and visualized under UV light with an Alpha Imager HP System™ (ProteinSimple Inc., Santa Clara, USA) and the images were recorded by a Fluorchem 8900™ (ProteinSimple Inc.) .

Semi-quantitative Method

To compare the bacterial load in each sample, a semi-quantitative method was used following a protocol described elsewhere (Castillo et al., 2007). We determined the lower limit of detection of PCR experiments and this was defined as the smaller number of bacteria to be detected by the PCR method. To do this, twelve serial dilutions for each bacterial species were prepared using pure bacterial cultures standardized at an OD_{660} nm (optical density) of 1 with known cell concentrations (Figure 2.1). Each dilution for each bacterial species was amplified by PCR at specific conditions as described before. The amplified dilutions constituted a standard curve for each bacterial species.

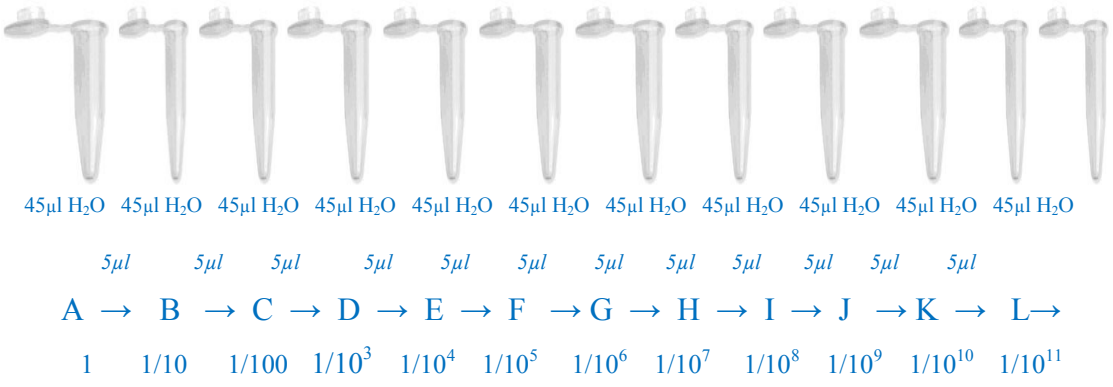

Figure 2.1: Serial dilutions for PCR Semi-quantitative Assessments

A: Pure culture of each bacterium.

According to intensity of the specific band obtained by PCR amplification and their number of cells, the samples were categorized into two categories: samples with a visible PCR band (positive or high number of cells) and samples with invisible PCR band (negative or low number of cells) (Appendix VII).

Table 2.2: Primer sequences for selected bacterial species

Bacterial species	DNA probe strains	Primer sequences (5' – 3')		References
Fusobacterium nucleatum	ATCC 25586	Fn-1 Fn-2	AGA GTT TGA TCC TGG CTC AG GTC ATC GTG CAC ACA GAA TTG CTG	(Fouad et al., 2002)
Prevotella intermedia	VPI 4196	BINT Pi	TCC GCA TAC GTT GCG TGC ACT CAA G CGT GCC AGC AGC CGC GGT AAT ACG	(Garcia et al., 1998)
Treponema denticola	ATCC 36405	Td-1 Td-2	TAA TAC CGA ATG TGC TCA TTT ACA T TCA AAGAAGCATTCCCTCTTCTTCTTA	(Ashimoto et al., 1996)
Porphyromas gingivalis	ATCC 33277	Pg F C11R	TGT AGA TGA CTG ATG GTG AAA ACC ACG TCA TCC CCA CCT TC	(Tran and Rudney, 1999)
Aggregatibacter actinomycetemcomitans	ATCC 43719	Aa F C11R	ATT GTT TAG CCC TGG TG ACG TCA TCC CCA CCT TCC TC	(Tran and Rudney, 1999)
Tannerella forsythia	ATCC 43037	Bf 392-1 Bf 392-2	ATG CTC CTG GGT CTG TT TCA AAGAAGCATTCCCTCTTCTTCTTA	(Guillot and Mouton, 1996)
Streptococcus mutans	NCTC 10449	Mutans F Mutans R	TCG CGA AAA AGA TAA ACA AAA CA GCC CCT TCA CAG TTG GTT AG	(Psoter et al., 2011)

2.6: Statistical Analysis

In order to obtain frequency counts, percentages, and univariate means, the data were first subjected to descriptive statistical tests. Normality of data distribution was assessed with the Shapiro-Wilk test. Fisher's exact test and one way-ANOVA test were used to detect between-groups differences in socio-demographic characteristics, prevalence of caries, oral levels of bacterial pathogens, and clinical parameters. Between-groups differences in decayed surfaces were analyzed using Kruskall-Wallis test followed by Mann-Whitney U test. Oral levels of *S. mutans* were compared to periodontal pathogens levels within subjects using Fisher's exact test. Bonferroni corrections were conducted for pairwise comparisons. We calculated unadjusted odds ratios and their 95% confidences intervals, to determine the strength of the association between explanatory and dependent variables. Statistical significance was set at $P < 0.05$. Data analyses were performed using IBM SPSS Statistics version 20 (IBM Corporation, Armonk, USA) and SAS 9.2 (SAS Institute, Cary, USA).

2.7: Bioethical Considerations

The ethics committee of the Université de Montréal (CÉRSS) approved the study. Written consent forms were signed by all of the participants after being informed about the study procedures.

To ensure confidentiality, a dental record number written on the digital questionnaires clinical records and vials containing the plaque samples for identification purposes identified each participant. A third party replaced the dental number with a new study code before sample analysis. All participant codes were recorded in a database and kept by a third party to ensure masking of the investigators.

CHAPTER THREE

Results

3: Results

3.1: Manuscript

Do individuals with chronic periodontitis have more caries than those with a healthy periodontium? *

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Key words: periodontal disease, caries, *Streptococcus mutans*, *Porphyromonas gingivalis*, *Treponema denticola*

Abstract

Aim: The aim of this clinical study was to compare adults with a healthy periodontium and those with chronic periodontitis, in terms of caries' risk and caries' clinical and microbiological measures.

Methods: Ninety-six healthy adults were divided into chronic periodontitis (n= 38) and healthy periodontium (n=58) based on their periodontal status, and matched for age, gender, and ethnic background. Chronic periodontitis was defined as having at least four teeth with ≥1 site with a pocket depth ≥4 mm and clinical attachment loss ≥2 mm. Each group were subsequently subdivided in 2 groups according to their caries status: participants having at least one untreated decayed surface and those with no untreated caries. Data were collected by means of self-administrated questionnaire, clinical examination, and supra- and subgingival plaque sampling. Assessments of oral levels of *Streptococcus mutans* and six periodontal pathogens were conducted by PCR amplification techniques. Data were analyzed using descriptive and bivariate statistical tests.

Results: Individuals with chronic periodontitis were 3.5 times more likely to have caries than healthy individuals (OR 3.5; CI: 1.5 – 8.3; $P = 0.006$). Subjects with both chronic periodontitis and dental caries had a significantly lower level of education than periodontally healthy subjects without dental caries (OR 6.0; CI: 1.7 – 21.7; $P = 0.04$). A significant higher proportion of subjects with high oral levels of *Porphyromonas gingivalis (P. g.)* and *Treponema denticola (T. d.)* was found among subjects with chronic periodontitis and untreated caries compared to periodontally healthy subjects with untreated caries (*P. g.*: OR 8.6; CI: 2.4 – 30.3; $P = 0.004$ and *T. d.*: OR 10.0; CI: 2.6 – 38.1; $P = 0.003$).

Conclusion: The results of this study suggest that adults with chronic periodontitis are more prone to caries disease than those adults with a healthy periodontium. Furthermore, low educational level could have a negative impact on the periodontal status of individuals.

Introduction

Periodontal diseases and dental caries represent major public health problems worldwide (Petersen and Ogawa, 2005). The two diseases affect the majority of the population in most industrialized and underdeveloped countries (Petersen et al., 2005; Pihlstrom et al., 2005).

Although common risk factors such as poor oral hygiene are associated with periodontal diseases and dental caries, the specificity of their causal microorganisms suggests distinct pathogenic mechanisms (Kinane et al., 1991). *Streptococcus mutans* is a major microorganism leading to initiation of dental caries (Selwitz et al., 2007). Periodontal diseases are principally caused by a group of Gram-negative anaerobic bacteria including *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Fusobacterium nucleatum*, *Prevotella intermedia*, and *Aggregatibacter actinomycetemcomitans* (Socransky et al., 1998; Holt and Ebersole, 2005; Pihlstrom et al., 2005; Darveau, 2010; Curtis et al., 2011).

This difference in microbiological pathogens could explain the lack of association observed between these two oral diseases in several studies (Miller and Seidler, 1940; Brucker, 1943; Massler et al., 1952; Kinane et al., 1991). However, a number of investigators have reported a positive association between these two prominent oral diseases (Massler and Sen Savara, 1951; Vehkalahti and Paunio, 1994; Albandar et al., 1995; Ekstrand et al., 1998; Saotome et al., 2006), and suggested that individuals suffering from periodontitis are more prone to caries than those with healthy periodontium. Furthermore, according to the study by Fine et al. (Fine et al., 2007). *A. actinomycetemcomitans*, a periodontal pathogen, has an inhibitory effect on the growth of *S. mutans* in individuals with aggressive periodontitis. This finding supports the hypothesis that there is a reverse association between caries and periodontal diseases as found by other clinical studies (Ramfjord, 1961; Fine et al., 1984; Sewon et al., 1988; Sioson et al., 2000; Iwano et al., 2010).

These controversial findings suggest that there is a need to further investigate the association between these two common oral diseases. Therefore, in this study we tested the hypothesis that there is no difference between individuals with a healthy periodontium and those with chronic periodontitis in terms of caries' risk and caries' clinical and microbiological measures.

Material and methods

Study design and participants

In this clinical study, 96 participants were recruited from the Université de Montréal and McGill University dental clinics, and advertisement in a local newspaper. Subjects were eligible to participate in the study if they were at least 30 years old, and were excluded from the study if they had fewer than 20 natural teeth, were diagnosed with aggressive periodontitis, had any contraindication to dental examination due to a medical condition, had taken antibiotics or had a dental cleaning 3 months prior to study procedures, required antibioprophylaxis prior to dental examination, had any untreated acute oral infection, or were unable to undertake study procedures or provide informed consent.

The participants were divided into two groups based on their periodontal status. Thirty-eight participants were diagnosed as having chronic periodontitis and 58 subjects had a healthy periodontium. Subgroup analysis was performed after classifying each group into two subclasses according to their caries status: participants having at least one decayed surface and those with no caries. The Université de Montréal ethics committe approved the protocol for this study and informed written consent was obtained from each participant.

Data collection

Data collection consisted of clinical and microbiological assessments as well as a self-administrated questionnaire on socio-demographic characteristics, medical and dental history, and smoking habits. All clinical and microbiological examinations were performed by a trained and calibrated general dentist (AR).

Clinical examination

The clinical examination consisted in diagnosing caries and periodontal status by using a mouth mirror, an explorer (EXD57, Hu-Friedy Mfg Co., Chicago, USA), and a manual periodontal probe (PCPUNC156, Hu-Friedy Mfg Co.). Subjects having at least 4 teeth with ≥1 site with a pocket depth ≥4 mm and a clinical attachment loss ≥2 mm were considered as patients with chronic periodontal disease (Michalowicz et al., 2006). Radike's criteria were used to diagnose coronal and root caries (Radike, 1972).

The components of the clinical examination were assessment of bleeding on probing (Carter and Barnes, 1974), plaque score (O'Leary et al., 1972), probing depth (PD), and clinical attachment level (Ramfjord, 1967) on the mesio-buccal, mid-buccal, and disto-lingual aspects of two randomly selected maxillary and mandibular quadrants of the dentition, excluding third molars. It was demonstrated that this partial recording protocol had the lowest bias for the prevalence of periodontal disease and its severity (Susin et al., 2005; Kingman et al., 2008). Clinical caries measurement consisted of a full mouth examination (except third molars) and the report of the decayed, missing, and filled surfaces (DM_3FS) index (Broadbent and Thomson, 2005).

Microbiological investigation

In order to investigate the microbiological nature of the patient's dental plaque, supragingival plaque samples were collected from four supragingival restoration/caries-free tooth surfaces located in the four quadrants of the mouth. Once the supragingival plaque had been removed with a cotton swab, subgingival plaque samples were collected from eight periodontal pockets (PD ≥ 5 mm) located in the four quadrants of the mouth to identify periodontal species. Then, plaque samples were immersed in separated tubes containing 500 µl of phosphate buffered saline (PBS, pH 7.0). After being sonicated and mixed in a vortex, they were immediately stored at -80°C for subsequent analyses.

The presence of targeted microorganisms was investigated by polymerase chain reaction (PCR), using specific primers according to the literature (Ashimoto et al., 1996; Guillot and Mouton, 1996; Garcia et al., 1998; Tran and Rudney, 1999; Fouad et al., 2002; Psoter et al., 2011). These

microorganisms included the cariogenic pathogen *Streptococcus mutans* and the periodontal pathogens *Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia, Treponema denticola, Tannerella forsythia,* and *Aggregatibacter actinomycetemcomitans*. The PCR conditions were those previously described for each bacterial species (Ashimoto et al., 1996; Guillot and Mouton, 1996; Garcia et al., 1998; Tran and Rudney, 1999; Fouad et al., 2002; Psoter et al., 2011). Briefly, PCR reagents used for all bacteria included a PCR buffer containing 200 mM of Tris-HCL (pH = 8.4), 500 mM of KCL, $MgCl_2$ (Invitrogen®, Life Technologies Co), a standard Taq buffer (Taq PCR Kit®, New England Biolabs Inc., Ipswich, USA), and 10 mM dNTPs (Invitrogen®, Life Technologies Co., Carlsbad, USA). All PCR amplification procedures were performed in a thermal cycler (PE2400®, Life Technologies Co.). The amplification products were analyzed on 2.5% agarose gel electrophoresis in tris-acetate ethylenediaminetetraacetic acid (EDTA) buffer (TAE) (40mM Tris-acetate, 1 Mm of EDTA at pH 8.3 and ethidium bromide [0.7ug/ml]). The amplification products were visualized and photographed under a UV light transilluminator (Alpha Imager®, Protein Simple Co., Santa Clara, USA) and the images were recorded (Fluorchem 8900®, Protein Simple Co.). The photographs of the gels were scored for the presence or absence the selected bacteria amplicons.

Statistical analysis

In order to obtain frequency counts, percentages, and univariate means, the data were first subjected to descriptive statistical tests. Normality of data distribution was assessed with the Shapiro-Wilk test. Fisher's exact test and one way-ANOVA test were used to detect between-groups differences in socio-demographic characteristics, prevalence of caries, oral levels of bacterial pathogens, and clinical parameters. Between-groups differences in decayed surfaces were analyzed using Kruskall-Wallis test followed by Mann-Whitney U test. Bonferroni corrections were conducted for pairwise comparisons. Odds ratios and their 95% confidences intervals were calculated to determine the strength of the association between explanatory and dependent variables. Statistical significance was set at $P < 0.05$. Data analyses were performed using IBM SPSS Statistics version 20 (IBM Corporation, Armonk, USA) and SAS 9.2 (SAS Institute, Cary, USA).

Results

All 96 participants completed the study procedures. The sample was comprised of men and women in a 1:1 ratio. The mean age of the sample population was 55.4 (SD ± 13.0) years. Overall, thirty-eight percent of the sample population was living alone, 24% had an education level of high school or less, 68% were born in Canada, and 56% had an annual income of less than $30,000 (data not shown). No statistically significant differences in socio-demographic variables were found between the four subgroups, except for educational level (Table 3.1). Subjects with chronic periodontitis had statistically significant lower level of education than healthy controls (OR 5.2; CI: 1.9 – 14.5; $P = 0.001$).

In individuals with healthy periodontium, 34 had no untreated caries (group A) and 24 had at least one untreated decayed surface (group B). Within participants with chronic periodontitis, 11 had no untreated caries (group C) and 27 had untreated caries (group D). Individuals with chronic periodontitis were 3.5 times more likely to have untreated caries than individuals with healthy periodontium (OR 3.5; CI: 1.5 - 8.3; $P = 0.006$) (Figure 3.1). No statistically significant differences were detected between subgroups for bleeding on probing, plaque index, DMFS, and DFS indices (Table 3.2).

Figure 3.2 demonstrates the example of semi-quantitative PCR for *P. gingivalis.* The PCR bands in lanes no. 1 to 7 counted as positive or high number of cells and PCR bands of lanes no. 8 and 9 were considered as negative or low number of cells. The same method was used for all other bacteria. Table 3.3 shows oral levels of *S. mutans* and periodontal pathogens per study group. There were significantly higher proportions of subjects with chronic periodontitis and untreated caries (group D) with high oral levels of *P. gingivalis* and *T. denticola* than in healthy subjects with untreated caries (group B) (*P. g.*: OR 8.6; CI: 2.4 – 30.3; $P = 0.004$ and *T. d.*: OR 10.0; CI: 2.6 – 38.1; $P = 0.003$). None of the participants in the healthy groups (A or B) demonstrated high levels of *P. intermedia* whereas a bigger proportion of participants with chronic periodontitis had high levels of this pathogen ($P > 0.05$). No statistically significant between-groups differences in oral levels of *S. mutans*, *F. nucleatum, T. forsythia, P. intermedia*, and *A. actinomycetemcomitans* were detected. Table 3.4 demonstrates that subjects with high levels of *S. mutans* were more susceptible to have high levels of *F. nucleatum* and *A.*

actinomycetemcomitans (*F .n.*: OR 6.4; CI: 1.6-24.6; *P* = 0.007 and *A. a.*: OR 2.5; CI: 1.1 - 6.0; P = 0.05). Proportions of subjects with high oral levels of *P. gingivalis*, *T. denticola, P. intermedia,* and *T. forsythia* were not significantly different between subjects with high and low levels of *S. mutans*.

Discussion

In this study, we tested the hypothesis that there is no difference between individuals with a healthy periodontium and those with chronic periodontitis in terms of caries' risk and caries' clinical and microbiological measures. We found that individuals with chronic periodontitis were more prone to have untreated caries and there was no association between oral levels of *S. mutans* with the majority of studied periodontal pathogens. However, we found that subjects with high oral levels of *S. mutans* were significantly more susceptible to have high levels of *F. nucleatum* compared to individuals with low levels of *S. mutans*, regardless of their caries or periodontal status.

Our study supports the hypothesis that there is a positive association between prevalence of chronic periodontitis and the frequency of caries in adults, as reported by other groups of investigators (Vehkalahti and Paunio, 1994; Saotome et al., 2006). Vehkalahti and Paunio reported a positive correlation between signs of periodontitis and number of decayed, filled, and missing teeth after examining 4,777 Finnish adults aged 30 years and older. In that study, sex difference was found to be related to this association. Male patients with deeper pocket depth demonstrated a higher frequency of root caries. In another study, poor oral hygiene was reported as a factor explaining the positive association of caries and periodontal disease (Kelbauskas et al., 2003). However, in our study, oral hygiene and sex were not determinants of this association. This could be explained by different factors. In the present study, oral hygiene was defined by a dichotomous plaque index. Thus, the cross-sectional design of the study could have resulted in a measurement bias. Furthermore, participants were mostly recruited from university clinics and were aware of oral hygiene benefits, which could have led to selection bias. However, participants with chronic periodontitis and untreated caries had a lower level of education compared to healthy controls having no untreated caries. In large national surveys, the level of

education has been associated with periodontal treatment needs and oral hygiene, which could explain our study finding on this issue (Dye and Vargas, 2002; Hermann et al., 2009).

In the present study, we did not find any between-groups differences in term of oral levels of *S. mutans*. This is in contrast with a recent study in which higher salivary levels of this pathogen were found in subjects with untreated caries regardless of their periodontal status (Iwano et al., 2010). This can be explained by the potential difference in population and sample collection methods. In fact, in the present study, supragingival plaque samples were collected mostly from the buccal surfaces of teeth. Because plaque control tends to be more efficient on buccal than lingual tooth surfaces (Lee and Moon, 2011; Malekafzali et al., 2011; Prasad et al., 2011), it is possible that oral levels of *S. mutans* were underestimated. One way to overcome this limitation might have been to measure salivary levels of bacteria (Umeda et al., 1998; Hong and Hu, 2010). However, with standard PCR techniques, a significant amount of plaque is required to detect a pathogen and this could not have been realized through saliva sample collection.

In the present study and in accordance with other authors, high oral levels of *P. gingivalis* and *T. denticola* were associated with chronic periodontitis (Holt and Ebersole, 2005; Brennan et al., 2007; Saygun et al., 2011). Furthermore, we found that *A. actinomycetemcomitans* species had a low frequency in patients with chronic periodontitis, as reported by other investigators (Zambon, 1985a; Fine et al., 2007; Saygun et al., 2011). On the other hand, we did not observe any association between *F. nucleatum, T. forsythia,* and *P. intermedia* and periodontal disease, which is contrary to the findings of other investigators (Darout et al., 2003; Saygun et al., 2011). This discrepancy could be due to different populations, clonal types of bacteria, bacterial detection methods, and the severity of periodontal disease (Papapanou et al., 2002). In the present study, standard PCR amplification technique was used to detect bacterial species. In fact, this technique has been reported to be less sensitive and have lower resolution than real-time PCR (qPCR) technique (Schmittgen, 2001; Maurer, 2011; Psoter et al., 2011). However, for logistical reasons, the qPCR technique was not available in this study.

Within the limits of this study, adults with chronic periodontitis seem to have higher frequency of untreated caries than individuals with a healthy periodontium. Furthermore, a low education level and high oral levels of *P. gingivalis* and *T. denticola* were associated with chronic periodontitis in adults. Future research is warranted to examine the association between caries

and periodontal disease, to determine their potential microbiological interactions, and to assess other effective variables such as time elapsed since the last dental visit.

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Table 3.1 ─ Socio-demographic Characteristics by Subgroups

	Subjects with healthy periodontium (n=58)		Subjects with chronic periodontitis (n=38)		
Variables	**Group A** Caries-free[1] (n=34)	**Group B** With caries (n=24)	**Group C** Caries-free (n=11)	**Group D** With caries (n=27)	***P*- Value* of significant pairwise comparisons**
Mean age (±SD)	52.5 ± 13.7	58.3 ± 12.9	56.6 ± 13.1	55.9 ± 12.0	
Female (%)	35.3	62.5	54.5	55.6	
Ethnicity (%) North American European Others[2]	 64.7 14.7 20.6	 70.8 16.7 12.5	 72.7 9.1 18.2	 74.1 14.8 11.1	
Education (%) High school or less	11.8	13.0	36.4	44.4	AD: 0.044
Living status (%) Alone	26.5	45.8	40.0	46.2	
Income (%) Less than $30,000	50.0	52.6	37.5	75.0	
Diabetes (%) Yes	2.9	8.3	9.1	7.4	
Tobacco use (%) Yes	14.7	12.5	18.2	14.8	

[1] Caries status is determined by the presence or absence of untreated caries lesions.
[2] Included Asians, Middle Eastern individuals, and Africans.
* All *P* values calculated using Fisher's exact tests except for mean age, which was calculated with one-way ANOVA.

Table 3.2 ─ Clinical Parameters by Subgroups

	Subjects with healthy periodontium (n=58)		**Subjects with chronic periodontitis (n=38)**		
Variables (mean ± SD)	**Group A** **Caries-free[1] (n=34)**	**Group B** **With caries (n=24)**	**Group C** **Caries-free (n=11)**	**Group D** **With caries (n=27)**	***P*- Value* of significant pairwise comparisons**
Bleeding on probing (% sites)	28.0 ± 20.2	24.9 ± 21.1	27.5 ± 18.1	31.5 ± 17.8	
Plaque index (% sites)	59.6 ± 15.6	63.4 ± 24.5	65.3 ± 18.1	70.4 ± 20.8	
Pocket depth (mm)	2.3 ± 0.2	2.2 ± 0.3	2.9 ± 0.4	2.8 ± 0.4	AC, AD, BC, BD: < 0.001
Attachment level (mm)	1.9 ± 0.4	2.1 ± 0.5	2.8 ± 0.9	2.8 ± 0.9	AC, AD, BD: < 0.002 BC: 0.027
DMFS	34.3 ± 19.3	43.2 ± 21.5	44.3 ± 27.2	47.7 ± 25.2	
DFS	27.0 ± 16.8	34.2 ± 18.1	37.7 ± 27.0	38.5 ± 23.5	
DS[2]	0	2.5 (1-14)	0	3.0 (1-21)	AB, AD, BC, CD: < 0.001

[1] Caries status is determined by the presence or absence of untreated caries lesions.

[2] DS is represented with median (min-max) rather than mean ± SD since 34 of the 58 healthy subjects had 0 DS.

* *P*- value calculated using one-way ANOVA except for median DS, which was calculated using Kruskal-Wallis and Mann-Whitney U tests. Statistical significance set at $P < 0.05$ for comparisons of subgroups after Bonferroni corrections.

Table 3.3 ─ Microbiological Profiles by Subgroups

	Subjects with healthy periodontium (n=58)		**Subjects with chronic periodontitis (n=38)**		
Oral levels of bacteria (% of subjects)	**Group A** **Caries-free[1] (n=34)**	**Group B** **With caries (n=24)**	**Group C** **Caries-free (n=11)**	**Group D** **With caries (n=27)**	***P*- Value* of significant pairwise comparisons**
Streptococcus mutans -High -Low	41.2 58.8	50 50	45.5 54.5	37 63	
Fusobacterium nucleatum -High -Low	8.8 91.2	25 75	9.1 90.9	14.8 85.2	
Porphyromonas gingivalis -High -Low	41.2 58.8	25 75	63.6 36.4	74.1 25.9	BD: 0.004
Prevotella intermedia -High -Low	0 100	0 100	9.1 90.9	14.8 85.2	
Treponema denticola -High -Low	35.3 64.7	16.7 83.3	54.5 45.5	66.7 33.3	BD: 0.003
Tannerella forsythia -High -Low	79.4 50.6	87.5 12.5	90.9 9.1	88.9 11.1	
Aggregatibacter actinomycetemcomitans -High -Low	32.4 67.6	33.3 66.7	45.5 54.5	33.3 66.7	

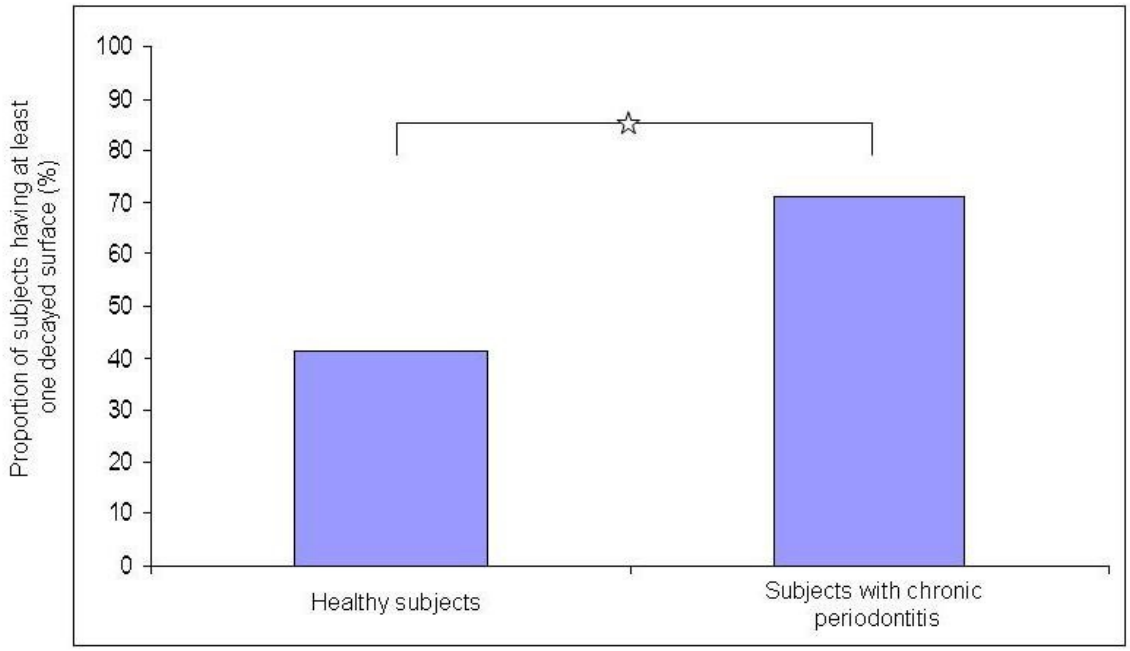
[1] Caries status is determined by the presence or absence of untreated caries lesions.

* All *P*-values calculated using Fisher's exact test. Statistical significance set at $P < 0.05$ for comparisons of subgroups after Bonferroni corrections.

Table 3.4 ─ Microbiological Profiles According to Oral Levels of *S. mutans*

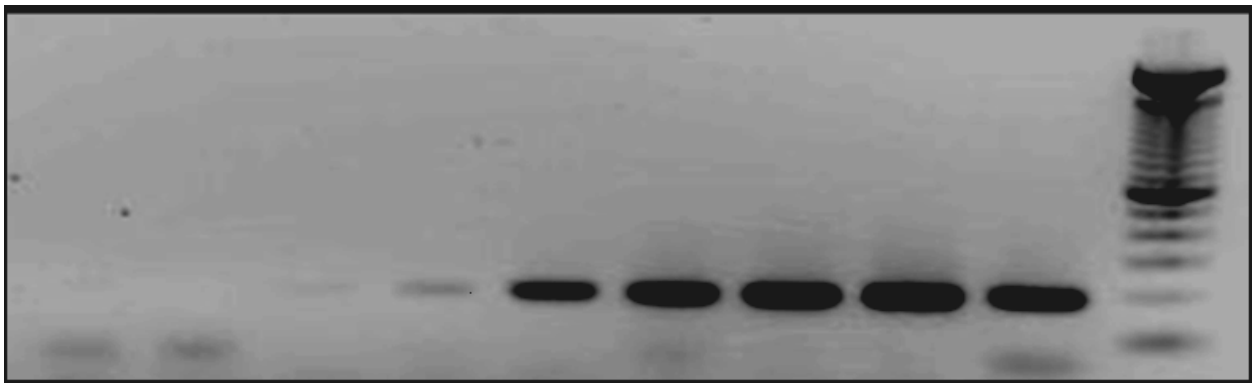
Oral levels of periodontal pathogens (% of subjects)	**Subjects with high level of *S. mutans* (n=41)**	**Subjects with low level of *S. mutans* (n=55)**	***P*-value***
Fusobacterium nucleatum -High -Low	26.8 73.2	5.5 94.5	0.007
Porphyromonas gingivalis -High -Low	48.8 51.2	49.1 50.9	1.00
Prevotella intermedia -High -Low	2.4 97.6	7.3 92.7	0.39
Treponema denticola -High -Low	41.5 58.5	41.8 58.2	1.00
Tannerella forsythia -High -Low	87.8 12.2	83.6 16.4	0.77
Aggregatibacter actinomycetemcomitans -High -Low	46.3 53.7	25.5 74.5	0.05

* All *P* values calculated using Fisher's exact tests.



P-value calculated using Fisher's exact test.
☆ $P = 0.006$.

Figure 3.1: Percentage of Individuals with Caries According to Their Periodontal Status



9 8 7 6 5 4 3 2 1

Figure 3.2: Example of PCR Semi-quantitative Assessment of *P. gingivalis*

1-7 = High number of cells, > 7 = Low number of ce

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CHAPTER FOUR

DISCUSSION

4: Discussion

This cross-sectional study examined the potential association between caries and chronic periodontitis in adults. A positive association was found between the frequency of caries and chronic periodontitis. Low educational level and high levels of *P. gingivalis* and *T. denticola* bacteria were found to be associated with chronic periodontitis in this population. There were no differences in caries indices and oral levels of *S. mutans* between healthy subjects and those with chronic periodontitis. Moreover, no association was found between oral levels of *S. mutans* and six periodontal pathogens.

To our knowledge, this is the first clinical study in which association between dental caries, chronic periodontitis, and their microbiological etiologic factors has been studied in an adult population. Previous investigations focused either on clinical signs of caries and chronic periodontitis or oral levels of a limited number of bacteria. In this cross-sectional study, socio-demographic and clinical data were analyzed and oral levels of seven well-known pathogens were determined, thus offering the possibility to examine microbiological profiles in relation to caries and periodontal status in a sample of the Quebec adult population.

Our study supports the hypothesis that there is a positive association between prevalence of dental caries and periodontal diseases as reported by other investigators (Vehkalahti and Paunio, 1994; Albandar et al., 1995). Vehkalahti and Paunio examined the presence or absence of gingival inflammation, periodontal pocket depth, and sub-gingival calculus in 4,777 Finnish adults aged 30 years and older and reported a positive correlation between periodontal status and number of decayed, filled, and missing teeth (Vehkalahti and Paunio, 1994). Male patients with deeper pocket depth demonstrated a significantly higher frequency of root caries. The positive correlation between caries and periodontal disease has been reported in young individuals as well. Albandar et al. did a series of examinations in young Brazilian adolescents to determine the association between untreated proximal caries and periodontal attachment loss (Albandar et al., 1995). Bite-wing radiographs of 227 thirteen-year-old subjects were used to assess proximal caries and periodontal disease in that study. The authors concluded that presence of proximal manifest caries was a risk factor for periodontal attachment loss. Also, presence of an interproximal restoration was significantly associated with alveolar bone loss, independently of whether the restoration was adequate or not. In another study, the positive association

between caries and periodontal disease was explained by the presence of poor oral hygiene (Kelbauskas et al., 2003). However, in the present study, oral hygiene and sex were not the determinants of this association. This could be explained by the fact that oral hygiene was determined by the plaque index at the time of dental examination.

In the present study, there was a statistically significant association between low level of education and presence of both chronic periodontitis and dental caries. According to the literature, the level of education influences the level of oral hygiene and periodontal treatment needs (Dye and Vargas, 2002; Hermann et al., 2009). This association could explain our study findings on this issue. It is generally accepted in the medical and public health literature that individuals with lack of education tend to have less access to healthcare than those with high education level (Millar and Locker, 1999; Public Health Agency of Canada, 2008; Mikkonen and Raphael, 2010), which favours the risk of having poor oral hygiene.

Several studies did not find any association between caries and periodontal diseases (Miller and Seidler, 1940; Massler et al., 1952; Kinane et al., 1991). Miller and Seidler studied 1,003 subjects who had at least 16 teeth present, with nearly the same percentage of men and women (Miller and Seidler, 1940). The radiographs of subjects were used to collect clinical data. Periodontal disease was defined according to presence or absence of radiographic interproximal bone loss. Moreover, missing number of teeth of each subject was recorded as caries status. In their study, men were more susceptible to periodontal disease compared to women who demonstrated more caries. The investigators explained the lack of correlation between caries and periodontal disease by the sex-difference prevalence of these two diseases. However, the number of decayed and filled teeth was not determined and no clinical periodontal examination was performed. In addition, the study by Kinane and colleagues also used radiographic bone loss as a definition of periodontal disease but did not include clinical parameters (Kinane et al, 1991). Therefore, it is possible that these two studies might have underestimated the prevalence of periodontitis since radiographic examination will show bone loss only once 30–50% or more demineralization has occurred (Jeffcoat et al., 1995). Massler and coworkers studied white male subjects aged 17–20 years to examine the correlation between caries and periodontal disease (gingivitis) (Massler et al., 1952). The number of decayed, missing, and filled teeth and surfaces (DMFS and DMFT) was recorded as caries status. Periodontal examination included the number of papillary, marginal, and attached gingival areas that were affected in the mouth. It is important to

underline that the Miller and Massler studies included young subjects (less than 21 years old), in whom chronic periodontitis is known to have a very low prevalence (Califano, 2003). Consequently, the authors most likely measured presence or absence of gingivitis in their study populations rather than periodontitis.

A number of studies indicated a reverse relationship between caries and periodontal diseases (Fine et al., 1984; Sewon et al., 1988; Sioson et al., 2000; Iwano et al., 2010). Fine et al. examined 13 patients with aggressive periodontitis and 10 patients with a healthy periodontium. The number of decayed, filled, and missing tooth surfaces (DMFS) was used to evaluate the clinical caries status, including radiographs to detect interproximal caries (Fine et al., 1984). The authors did not find any significant difference in the number of missing and filled surfaces between aggressive periodontitis subjects and controls. However, controls had significantly more proximal caries compared to aggressive periodontitis subjects and this is in contrast with our results. In addition, Sioson and colleagues (Sioson et al., 2000) studied the association between caries and aggressive periodontitis by comparing prevalence of proximal caries in subjects with aggressive periodontitis and matched healthy controls. They measured the DMFS index on radiographs and defined aggressive periodontitis as having bone loss on permanent first molars and/or incisors in 9- to 25-year-old subjects. The authors found that cases had significantly less proximal caries than controls. In adults, Sewon et al. examined the prevalence of caries in 1,011 Finnish subjects with severe periodontitis compared to 291 controls (Sewon et al., 1988). The prevalence of caries and periodontal disease was detected solely by orthopantomograms. They reported that adults with severe periodontitis had less caries compared to healthy individuals, which is in contrast with our results. These differences in the results between these three studies and our study might be explained by the fact that they included aggressive periodontitis (Fine et al., 1984; Sioson et al., 2000) and subjects with severe periodontitis (Sewon et al., 1988) in their studies, compared to our study in which cases were more likely to be early to moderate periodontitis subjects. However, although the sample size of the Sewon and colleagues study was significant, only proximal caries could be assessed since the study was based on panoramic radiographic assessments. A more recent study looked at the potential microbiological correlation between caries and periodontal diseases in Japanese adults (Iwano et al., 2010). The investigators studied salivary levels of *S. mutans* and *P. gingivalis* in relation to the periodontal and caries status. Forty subjects aged from 23 to 78 years participated in the study. Periodontal disease was defined as having a mean pocket depth ≥ 3mm. Subjects were divided in three groups: those having caries and no

periodontitis, those having periodontitis and caries, and those having periodontitis and no caries. No subject had a healthy periodontium and no caries. All subjects had a clinical examination including plaque and bleeding indices, pocket depth, and number of decayed surfaces. Salivary levels of *S. mutans* and *P. gingivalis* were evaluated by using real-time PCR methods. Thereafter, 10 periodontitis patients had scaling and root planing, and two to four months later, provided salivary samples to detect *S. mutans* and *P. gingivalis*. The authors found that the concentration of *P. gingivalis* significantly decreased after treatment while *S. mutans* levels increased. Also, clinical parameters of periodontal disease were significantly improved after therapy. Therefore, they concluded that there was an inverse relationship between caries and periodontal disease according to clinical and microbiological findings, which is in contrast with findings of our study. In the present study, we did not observed any association between the oral levels of *S. mutans* and oral levels of *P. gingivalis* and *T. denticola*. Iwano et al explained the reverse relationship between caries and periodontal disease by the effect of *S. mutans* on the pH level and therefore, an inhibitory effect on growth of *P. gingivalis*. The *P. gingivalis* bacterium is very sensitive and cannot survive in a pH below 6.5, and its growth is possible only over a pH range of 6.7–8.3 (Iwano et al., 2010). The discrepancy between this finding and our results could be due to the different methodologies, notably the use of real-time PCR for detection of bacteria, the collection of saliva instead of plaque samples, and the selection criteria for periodontitis subjects.

A number of studies suggested that carious lesions, restored or unrestored, could lead to the increase of gingival inflammation, attachment loss, and alveolar bone loss (Hakkarainen and Ainamo, 1980; Lang et al., 1983; Albandar et al., 1995). It was suggested that a positive association between caries and periodontal disease can be caused by physical retention of proximal plaque bacteria, or by local tissue irritation produced by the cavity, or by the restoration itself (Lang et al., 1983; Chen et al., 1987).

In the present study, we did not find any between-groups differences in terms of oral levels of *S. mutans*. This is in contrast with a recent study (Iwano et al., 2010). The authors found that in a Japanese adult population, there was a higher salivary level of *S. mutans* in subjects with caries, regardless of their periodontal status. This can be explained by the potential difference in population and sample collection methods. In fact, in the present study supragingival plaque samples were collected mostly from the buccal surfaces of teeth. Because plaque control tends to be more efficient on buccal than lingual tooth surfaces (Lee and Moon, 2011; Malekafzali et al., 2011; Prasad et al., 2011), it is possible that oral levels of *S. mutans* were underestimated. One way to overcome this limitation

could have been to measure salivary levels of bacteria (Umeda et al., 1998). However, in standard PCR techniques, a significant amount of plaque is required to detect a pathogen and this could not have been realized through saliva sample collection. On the other hand, the technique of PCR used in this study has been shown to be a sensitive and reliable technique for the amplification of specific DNA segments of bacteria in epidemiological studies (Franco e Franco et al., 2007). However, PCR technique can be influenced by the chemistry of the reaction mixture and the thermal conditions during logarithmic amplification. For instance, related mass of variability in the quality of primers and Taq polymerase as well as wrong manipulation of thermal cycles can result in failure of amplification reaction (Maurer, 2011). However, to ensure validity and precision of PCR results, initial and repeated calibrations were done for each bacterial species.

In this study and in accordance with other studies, high oral levels of *P. gingivalis* and *T. denticola* were associated with chronic periodontitis (Holt and Ebersole, 2005; Brennan et al., 2007; Saygun et al., 2011). Moreover, we found that *A. actinomycetemcomitans* species had low frequency in patients with chronic periodontitis. These results support the findings of previous work on the association between this pathogen and aggressive periodontitis rather than chronic periodontitis (Zambon, 1985b; Fine et al., 2007; Saygun et al., 2011). However, we did not observe any significant association between *F. nucleatum*, *T. forsythia*, and *P. intermedia* and periodontal disease, which is contrary to the findings of a number of investigators (Darout et al., 2003; Brennan et al., 2007; Saygun et al., 2011). These discrepancies could be due to different populations, clonal types of bacteria, bacterial detection methods, and the severity of periodontal disease. For example, a study from Thailand indicated a high prevalence of *T. forsythia* in subjects with severe periodontitis (Papapanou et al., 2002) while our Canadian study population had an early to moderate chronic periodontitis.

In the present study, high oral levels of *F. nucleatum* were associated with high oral levels of *S. mutans* but this finding was not extrapolated to other periodontal pathogens. Aggregation has a key role in interaction of bacteria in biofilm formation. For example, it was found *in vitro* that *S. mutans* can aggregate with *F. nucleatum* (Huang et al., 2011) and this could potentially explain the correlation between the levels of these two species. Furthermore, *S. mutans* have the ability to produce lactic acid and survive in a low pH environment. On the other hand, *P. gingivalis* is very sensitive and cannot survive in a pH below 6.5, and its growth is possible only over a pH range of 6.7–8.3 while *F. nucleatum* can grow in the wide range of pH from 5.0 to 7.0 (Iwano et al., 2010; Huang et al., 2011).

However, as the results from the Huang *et al* study are *in vitro*, clinical research with real quantification load for all studied bacterial species is warranted.

The lack of association between oral pathogens of caries and periodontal diseases and their clinical signs indicated that there might not be any significant microbial interactions between cariogenic and periodontal pathogens in the oral environment. Nevertheless, more attention should focus on subjects affected by periodontitis since attachment loss can result in root exposure to the oral environment and a more cariogenic flora. This can in turn lead to an increased susceptibility to caries, especially in elderly individuals, as illustrated by other authors (Saotome et al., 2006).

Limitations of the study

There were several limitations in this study. First, this study focused only on the cariogenic role of *S. mutans* while *lactobacillus* is known to be another prevalent cariogenic bacterium in coronal and root caries. More specifically, *S. mutans* is frequently isolated in higher concentrations in cavitated lesions (Brown et al., 1986), while *lactobacilli* species are found in higher proportions of advanced carious lesions (Byun et al., 2004). However, for logistical reasons, it was not possible to explore the association between oral levels of *lactobacilli* and clinical and microbiological parameters of periodontitis. Secondly, the low sensitivity and low resolution that can occur with the use of agarose gels during standard PCR amplification techniques may have limited the validity of our results. Ethidium bromide for staining electrophoresis gels is not quantitative and cannot detect less than tenfold among samples (Psoter et al., 2011). On the other hand, real-time quantitative PCR techniques (qPCR) can determine the amount of specific bacteria, being able to identify as little as twofold (Psoter et al., 2011). Thus, compared to standard PCR, qPCR would have been more precise and sensitive to detect the selected pathogens and the viability of the samples would not have had significant effects on the results (Kim, 2001; Schmittgen, 2001; Maurer, 2011). Thirdly, proximal caries were not assessed by means of radiographs. This could lead to measurement bias. However, the clinical DMFS score used in this study is still the most accepted caries assessment tool worldwide (Benigeri et al., 1998; Bloemendal et al., 2004; Hong et al., 2010). Finally, the small sample size of this study might have resulted in type II errors.

Future studies

Clinical studies with larger number of participants are needed to reach more conclusive results. Additional species of cariogenic bacteria like *lactobacilli* could be monitored and quantitative bacterial assessment methods could be used to determine the amount of pathogenic bacteria with increased precision. As there are a lot of variables that can have a potential effect on the association between caries and periodontal disease such as time elapsed since last dental visit, future studies assessing more potential confounding factors are needed.

CHAPTER FIVE

CONCLUSION

5: Conclusion

Within the limitation of this study, the results showed that each of these factors: frequency of caries, low education level, high oral levels of *P. gingivalis* and *T. denticola* have a significant positive association with the prevalence of chronic periodontitis in Quebec adults. There was no association between oral levels of periodontal pathogens and the oral levels of *S. mutans,* except for *F. nucleatum*. No association was found between oral levels of *S. mutans*, caries indices, and chronic periodontitis. Future research is warranted to explore further the relationship between caries and periodontal disease and to determine their potential microbiological interactions.

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APPENDICES

Appendix I: Consent form for participants with periodontal disease

Université de Montréal

The association between caries and periodontal diseases

(for participants with gum disease)

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Information and Consent Form

Introduction : About this study

We are asking you to participate to this research project because you have periodontal disease (gum disease). Before you accept to participate to this study, please take the time to understand and consider the following information.

This consent form explains to you the goal of this study, procedures, advantages, and the risks and inconvenients. The contact information of the people involved in this research project will be listed in case you would need to contact them.

The present form may contain words that you do not understand. We invite you to ask questions that you will judge pertinent to the researcher and the other staff members involved in this research project and to ask them any word or piece of information that is not clear to you.

From the moment you have this form, you will have about 15 minutes to give your approval and return it to us signed if you accept to participate to this study.

Study description

The investigators want to determine if there is an association between dental caries, the presence of bacteria in the mouth, and periodontal diseases (gum diseases). If there is an association, the investigators hope to understand the nature of such an association and the potential interaction between those 2 common oral diseases. About 150 men and women are expected to participate to this study and the participants will be divided in 2 groups: 1 group having periodontal diseases and the other not having periodontal diseases.

Nature of your participation and length of the study

If you want to volunteer to participate to this study, the following procedures will be undertaken in one appointment and will not require a follow-up appointment. This exam should take about 35-45 minutes and is done here on the dental chair. No radiograph will be taken as a part of this research project. A dentist-researcher:

- Will ask you a few questions concerning your medical history, your socio-economical situation and dental history.
- Will examine your teeth and gums. He/she will proceed to the same procedures that a dentist or hygienist does during a routine dental exam. He/she will use a small dental instrument to verify the presence or absence of caries by poking the dental surfaces. This should not hurt you. He/she will examine the gums around some of your teeth with a periodontal probe (small measuring instrument). This will be done so we can measure the depth of the space between your teeth and your gums and to see if your gums bleed when they are measured. You might be slightly uncomfortable and your gums might bleed if they are inflamed.
- Will collect 8 samples from the dental plaque around some of your teeth with an instrument. These samples will be kept on the side in small vials in order to detect later the concentration of bacteria causing dental caries and periodontal diseases. This sample technique might cause in certain cases minimal temporary gum soreness, especially if it is inflamed.

Conditions to participate

You can participate to this study if :

- You are between 30 and 65 years old.
- You are in relatively good health and do not have any contra-indication to dental treatments.

You cannot participate to this study if:

- You have less than 20 natural teeth in your mouth.
- You have an oral infection considered acute by your student/dentist.
- You are unable to give an informed consent.
- You are not able to cooperate with the study procedures.
- You have received scaling treatments (cleaning) during the past 3 months.
- You have taken antibiotics during the past 3 months.
- You have to take antibiotics before receiving dental treatments because you have, for example, heart murmurs or a joint prosthesis.

Risks and soreness

To diminish the risks of gum soreness, you can ask for the application of a topical gel to anesthesize or « numb » your gums. That gel is safe for pregnant or breastfeeding women. In some isolated cases, the participants could feel a slight temporary gum soreness (2-3 days) after having their teeth and gums examined. This is generally very uncommon since these procedures are those done during routine dental exams. If you feel so uncomfortable that you want to withdraw from the study, you can do it without penalty. If you have a soreness that bothers you, you can call the research director, Dr. Robert Durand, at the Faculté de Médecine Dentaire of the Université de Montréal. The researchers will report the secondary effects if they occur to the "comité d'éthique à la recnerché de l'Université de Montréal". However, no secondary effect is anticipated during this study.

Benefits of choosing to participate

You will learn if you have periodontal diseases and dental caries and if it is the case, we will refer you so you can receive appropriate treatments. To find and treat periodontal disease and caries can prevent the loss of your teeth. Moreover, results obtained could contribute to the advancement of knowledge in this field.

Voluntary participation and possibilities to withdraw

Your participation to this study is absolutely voluntary. You are therefore free to refuse to participate and this will not affect the quality of dental care that you will receive as a patient at the clinics of the Faculté de Médecine Dentaire. You can also withdraw from this study at any time without giving us any reason. You have to simply notify the research director or one of the research team members.

The research director can also terminate your participation if you do not respect the study protocol or if it is not in your own interest. On the other hand, the ethics committee (CÉRSS) can also stop the study, notably for security or feasibility reasons. In the event that you withdraw or are excluded, the data collected will be destroyed.

Confidentiality

During your participation to this project, the researcher and his/her team will collect in designated research file information concerning you in order to fulfill scientific objectives.

All the information collected will be strictly confidential. Since the dental record number of each participant will be written on the questionnaires, clinical exam records, and on the vials containing the plaque samples for microbial assessments, a third party (research assistant) will remove the medical record number stickers from the tubes before they are analyzed and place new stickers with a secret code. The

key to the code linking your name to your research file will be stored by that person in a locked office where only he/she has access to.

Personal and clinical informations and research results will be kept in a research file specifically created for this study. Microbiological samples will be kept in a freezer located in a microbiology laboratory at the Université de Montréal where only employees and researchers have access to.
Research data will be kept during 7 years after the study and will be destroyed thereafter.

You have the right to consult your research file to verify information collected and rectify them if needed as long as the principal investigator or the institution possess the information. However, in order to maintain the scientific integrity of this study, you might have access to certain information once your participation is finished.

For reasons of research surveillance and control, your research and dental files might be consulted by a person designated by the Université de Montréal ethics committee (CÉRSS). All these people will respect the confidentiality policy.

Data might be published in scientific journals but it will not be possible to identify you.

Compensation

You will receive an amount of 25$ to compensate for your transportation fees and your time.

By signing the present information and consent form, you do not renounce your rights nor free the researchers and the institution of their civil or professional responsibilities.

If you suffer from an injury or any lesion related to your participation to this study, you will receive treatments and services according to your health status without any fee from your part.

Communication of results

You will be able to communicate with the research team in order to obtain information on the progress of the procedures or results of the study. If you wish to know the study results, you have to contact us and there will be sent to you by email after the results have been published in a scientific journal.

Contacts

If you have questions about this study, you can communicate (before, during and after the study) with one of the following person :

- Dr. Robert Durand
- In the case of a medical emergency, communicate with the emergency of university.

For any question concerning the ethical aspect of your participation to this study, you can share your concern with the research director or explain your worries to the president of the ethics committee (CÉRSS), Mme Marie-France Daniel .

If you have questions concerning your rights as a study participant or if you have complaints or comments to formulate, you can contact the Université de Montréal ombudsman, Mme Pascale Descary .

The ethics committee ("Comité d'éthique de la recherche en sciences de la santé de l'Université de Montréal") has approved this study and ensure its monitoring. Moreover, it will approve any modification brought to the information and consent form and to the study protocol.

Consent

I have read and understood the information and consent form. I recognized that someone has explained to me the study, has answered my questions to my satisfaction, and that I have had enough time to take a decision. I consent to participate to this study according to the conditions listed above. A dated and signed copy of this information and consent form will be given to me.

Name (in printed letters) and signature of participant:

*Name:*_____

*Signature:*_____ *Date:*_____

Researcher's engagement and signature:

I certify that we have explained to the participant the terms of this information and consent form, that we have answered his/her questions concerning it, and that we have clearly indicated that he/she is free to withdraw from the study and this, without any negative consequence.

I engage myself along with the research team to respect what has been agreed upon the information and consent form and to give him/her a signed copy.

Name (in printed letters) and signature of principal investigator:

*Name:*_____

*Signature:*_____ *Date:*_____

Signature of the person obtaining the consent if different from principal investigator:

I have explained to the participant the terms of the present information and consent form and I have answered to the questions he/she asked me.

Name (in printed letters) and signature of the person obtaining the consent:

*Name:*_____

*Signature:*_____ *Date:*_____

Appendix II: Consent for healthy participants

Université de Montréal

The association between caries and periodontal diseases

(for participants with healthy gums)

Faculté de Médecine Dentaire
Université de Montréal

FONDS ERNEST - CHARRON

Research Director and Principal Investigator: Dr. Robert Durand, DMD, MS, FRCD(C)
Département de Santé Buccale
Faculté de Médecine Dentaire
Université de Montréal

Research Co-director : Dre. Elham Emami, DDS, MSc, PhD
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Student-researcher : Dre. Arezou Roufegarinejaad, DDS
Programme de Maîtrise en Sciences Bucco-dentaires
Département de Santé Buccale
Faculté de Médecine Dentaire
Université de Montréal

Information and Consent Form

Introduction : About this study

We are asking you to participate to this research project because you have healthy gums. Before you accept to participate to this study, please take the time to understand and consider the following information.

This consent form explains to you the goal of this study, procedures, advantages, and the risks and inconvenients. The contact information of the people involved in this research project will be listed in case you would need to contact them.

The present form may contain words that you do not understand. We invite you to ask questions that you will judge pertinent to the researcher and the other staff members involved in this research project and to ask them any word or piece of information that is not clear to you.

From the moment you have this form, you will have about 15 minutes to give your approval and return it to us signed if you accept to participate to this study.

Study description

The investigators want to determine if there is an association between dental caries, the presence of bacteria in the mouth, and periodontal diseases (gum diseases). If there is an association, the investigators hope to understand the nature of such an association and the potential interaction between those 2 common oral diseases. About 150 men and women are expected to participate to this study and the participants will be divided in 2 groups: 1 group having periodontal diseases and the other not having periodontal diseases.

Nature of your participation and length of the study

If you want to volunteer to participate to this study, the following procedures will be undertaken in one appointment and will not require a follow-up appointment. This exam should take about 35-45 minutes and is done here on the dental chair. No radiograph will be taken as a part of this research project. A dentist-researcher:

- Will ask you a few questions concerning your medical history, your socio-economical situation and dental history.
- Will examine your teeth and gums. He/she will proceed to the same procedures that a dentist or hygienist does during a routine dental exam. He/she will use a small dental instrument to verify the presence or absence of caries by examining the dental surfaces. This should not hurt you. He/she will examine the gums around some of your teeth with a periodontal probe (small measuring instrument). This will be done so we can measure the depth of the space between your teeth and your gums and to see if your gums bleed when they are measured. You might be slightly uncomfortable and your gums might bleed if they are inflamed.
- Will collect 8 samples from the dental plaque around some of your teeth with an instrument. These samples will be kept on the side in small vials in order to detect later the concentration of bacteria causing dental caries and periodontal diseases. This sample technique might cause in certain cases minimal temporary gum soreness, especially if it is inflamed.

Conditions to participate

You can participate to this study if :

- You are between 30 and 65 years old.
- You are in relatively good health and do not have any contra-indication to dental treatments.

You cannot participate to this study if:

- You have less than 20 natural teeth in your mouth.
- You have an oral infection considered acute by your student/dentist.
- You are unable to give an informed consent.
- You are not able to cooperate with the study procedures.
- You have received scaling treatments (cleaning) during the past 3 months.
- You have taken antibiotics during the past 3 months.
- You have to take antibiotics before receiving dental treatments because you have, for example, heart murmurs or a joint prosthesis.

Risks and soreness

To diminish the risks of gum soreness, you can ask for the application of a topical gel to anesthesize or « numb » your gums. That gel is safe for pregnant or breastfeeding women. In some isolated cases, the participants could feel a slight temporary gum soreness (2-3 days) after having their teeth and gums examined. This is generally very uncommon since these procedures are those done during routine dental exams. If you feel so uncomfortable that you want to withdraw from the study, you can do it without penalty. If you have a soreness that bothers you, you can call the research director, Dr. Robert Durand, at the Faculté de Médecine Dentaire of the Université de Montréal. The researchers will report the secondary effects if they occur to the "comité d'éthique à la recnerché de l'Université de Montréal". However, no secondary effect is anticipated during this study.

Benefits of choosing to participate

You will learn if you have periodontal diseases and dental caries and if it is the case, we will refer you so you can receive appropriate treatments. To find and treat periodontal disease and caries can prevent the loss of your teeth. Moreover, results obtained could contribute to the advancement of knowledge in this field.

Voluntary participation and possibilities to withdraw

Your participation to this study is absolutely voluntary. You are therefore free to refuse to participate and this will not affect the quality of dental care that you will receive as a patient at the clinics of the Faculté de Médecine Dentaire. You can also withdraw from this study at any time without giving us any reason. You have to simply notify the research director or one of the research team members.

The research director can also terminate your participation if you do not respect the study protocol or if it is not in your own interest. On the other hand, the ethics committee (CÉRSS) can also stop the study, notably for security or feasibility reasons. In the event that you withdraw or are excluded, the data collected will be destroyed.

Confidentiality

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key to the code linking your name to your research file will be stored by that person in a locked office where only he/she has access to.

Personal and clinical informations and research results will be kept in a research file specifically created for this study. Microbiological samples will be kept in a freezer located in a microbiology laboratory at the Université de Montréal where only employees and researchers have access to.
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For reasons of research surveillance and control, your research and dental files might be consulted by a person designated by the Université de Montréal ethics committee (CÉRSS). All these people will respect the confidentiality policy.

Data might be published in scientific journals but it will not be possible to identify you.

Compensation

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If you suffer from an injury or any lesion related to your participation to this study, you will receive treatments and services according to your health status without any fee from your part.

Communication of results

You will be able to communicate with the research team in order to obtain information on the progress of the procedures or results of the study. If you wish to know the study results, you have to contact us and there will be sent to you by email after the results have been published in a scientific journal.

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For any question concerning the ethical aspect of your participation to this study, you can share your concern with the research director or explain your worries to the president of the ethics committee (CÉRSS), Mme Marie-France Daniel .

If you have questions concerning your rights as a study participant or if you have complaints or comments to formulate, you can contact the Université de Montréal ombudsman, Mme Pascale Descary .

The ethics committee ("Comité d'éthique de la recherche en sciences de la santé de l'Université de Montréal") has approved this study and ensure its monitoring. Moreover, it will approve any modification brought to the information and consent form and to the study protocol.

Consent

I have read and understood the information and consent form. I recognized that someone has explained to me the study, has answered my questions to my satisfaction, and that I have had enough time to take a decision. I consent to participate to this study according to the conditions listed above. A dated and signed copy of this information and consent form will be given to me.

Name (in printed letters) and signature of participant:

*Name:*_____

*Signature:*_____ *Date:*_____

Researcher's engagement and signature:

I certify that we have explained to the participant the terms of this information and consent form, that we have answered his/her questions concerning it, and that we have clearly indicated that he/she is free to withdraw from the study and this, without any negative consequence.

I engage myself along with the research team to respect what has been agreed upon the information and consent form and to give him/her a signed copy.

Name (in printed letters) and signature of principal investigator:

*Name:*_____

*Signature:*_____ *Date:*_____

Signature of the person obtaining the consent if different from principal investigator:

I have explained to the participant the terms of the present information and consent form and I have answered to the questions he/she asked me.

Name (in printed letters) and signature of the person obtaining the consent:

*Name:*_____

*Signature:*_____ *Date:*_____

Appendix III : Socio-demographic questionnaire

SOCIODEMOGRAPHIC QUESTIONNAIRE for research project titled:
The association between caries and periodontal diseases

Date : ☐☐☐☐ / ☐☐ / ☐☐ (y y / m m / d d)

Chart Number/ Identification Code: ☐☐☐☐☐☐☐☐☐☐

Please answer the following questions.

Gender: ○ 1 Male ○ 2 Female

Native language: ○ 1 French ○ 2 English ○ 3 German ○ 4 Spanish ○ 5 Other :

Marital status: ○ 1 Single ○ 2 Married ○ 3 Separated ○ 4 Divorced ○ 5 Widow

Do you live: ○ 1 Alone? ○ 2 With family? ○ 3 With other adults? ○ 4 I prefer not to answer

Level of education : ○ 1 Elementary (7 years and less) ○ 2 High-school (8-12 years) ○ 3 College (13-15 years) ○ 4 University (16 years and more) ○ 5 I prefer not to answer

Present employment : ○ 1 Full time ○ 2 Part time ○ 3 At home ○ 4 Student ○ 5 Unemployed ○ 6 Retired ○ 7 I prefer not to answer

Annual household revenue: ○ 1 Less than 19 999$ ○ 2 Between 20 000 and 29 999$ ○ 3 Between 30 000 and 39 999$ ○ 4 Between 40 000 and 49 999$ ○ 5 Between 50 000 and 59 999$ ○ 6 Between 60 000 and 74 999$ ○ 7 more than 75 000$ ○ 8 I prefer not to answer

Appendix IV: Medical questionnaire

MEDICAL QUESTIONNAIRE ADDENDUM FOR RESEARCH PROJECT TITLED:
The association between caries and periodontal diseases

Date: ☐☐☐☐ / ☐☐ / ☐☐
y r m m d d

Chart number/ Identification Code ☐☐☐☐☐☐☐☐☐☐

Sex: ☐ M ☐ F

Date of birth: ☐☐☐☐ / ☐☐ / ☐☐
y r m m d d

Dental History

Do you need to take antibiotics prior dental treatments? ☐ YES ☐ NO

Have you had any scaling (dental cleaning) in the past 3 months? ☐ YES ☐ NO

Medical History

Have you taken any antibiotics in the past 3 months? ☐ YES ☐ NO

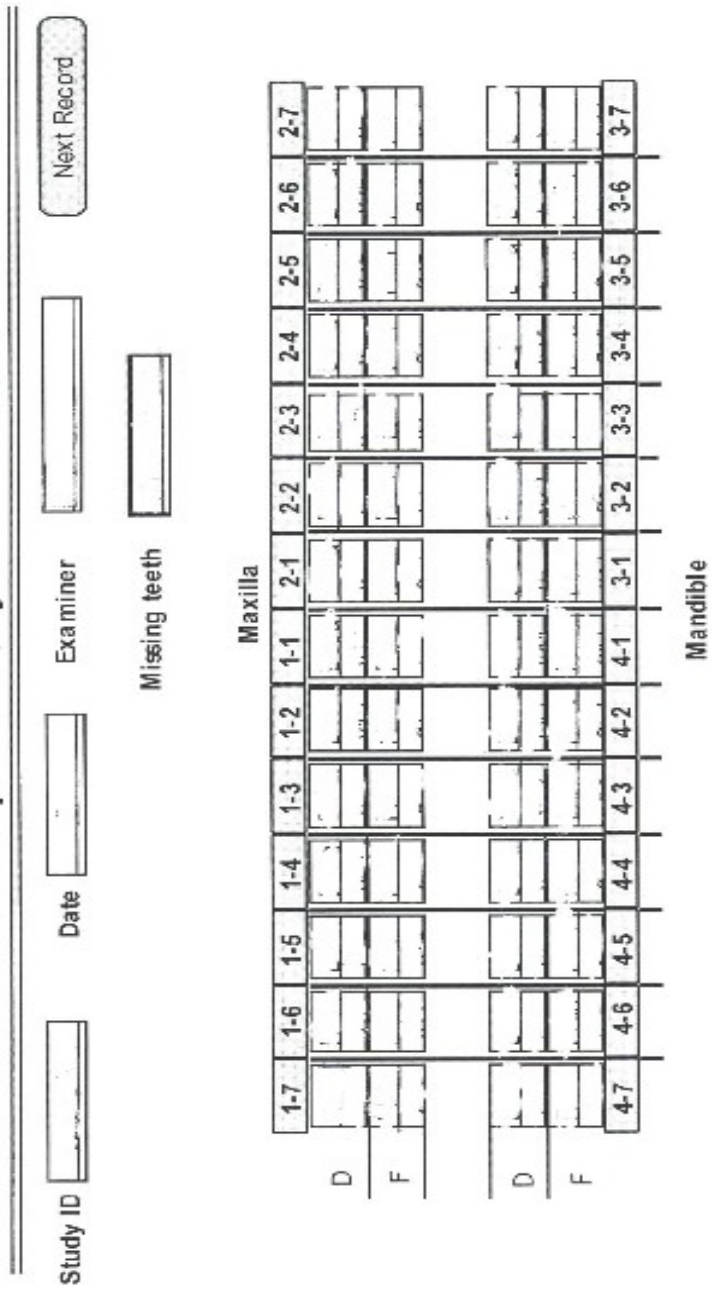
Do you suffer from diabetes? ☐ YES ☐ NO

If yes, which type: I ☐ II ☐

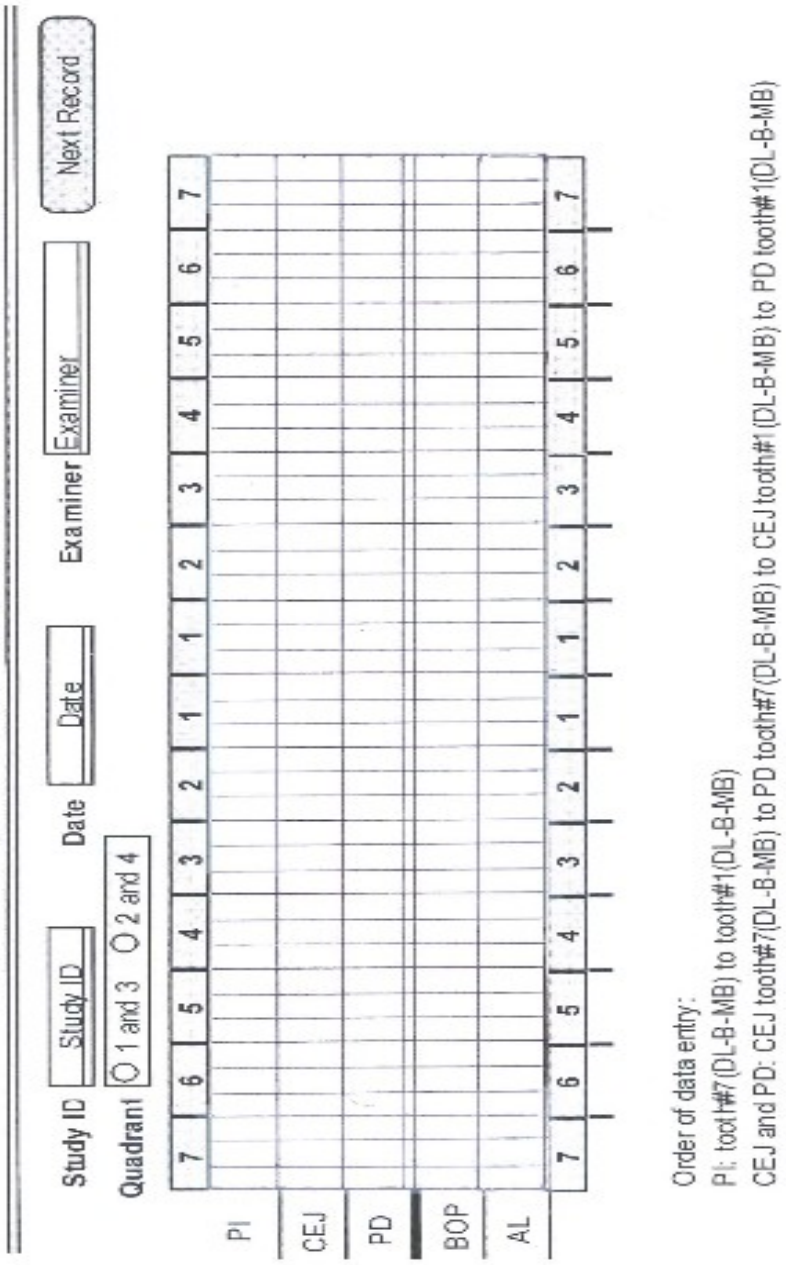
Do you use tobacco?: ○ 1 No
○ 2 Yes, _____ cigarettes / day
○ 2 Yes, _____ cigars / day
○ 2 Yes, _____ other tobacco use/ day

Have you ever used tobacco?: ○ 1 No
○ 2 Yes, within the past 6 months
○ 2 Yes, within the past year
○ 2 Yes, within the past 1 to 2 years
○ 2 Yes, within the past 2 to 5 years
○ 2 Yes, within the past 5 to 8 years
○ 2 Yes, longer than 8 years ago

Appendix V: Caries record form



Appendix VI: Periodontal record form



Appendix VII: Microbiological record form

Test vials monitoring/assessment sheet for research project titled *The.association between Caries and Periodontal Diseases*

Examiner	Date+Time	Dental Record Number / Study ID	Category of PCR samples High/ low
S. mutans			
F.nucleatum			
P. gingivalis			
P. intermedia			
T. denticola			
T. forsythia			
A.a			