

White Paper Report

Oral Infection—Systemic Disease Connection

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Oral Microbes and Systemic Disease

Over the last twenty plus years, dental disease has been reported to be associated with numerous systemic diseases¹, including, heart disease^{2 3 4 5 6 7 8 9}, atherosclerotic lesions^{10 11}, diabetes^{12 13}, and neurological diseases^{14 15 16 17 18 19 20 21 22 23}, brain abscesses²⁴, precancerous gastric lesions,²⁵ prostate cancer²⁶, and other cancers^{27 28 29}. Although there have been numerous theories as to why this relationship exists, causation remains elusive³⁰.

In this paper, we will attempt to describe the dilemma dentistry is facing when trying to mitigate the problems derived from evidence that contradict our basic understanding of dental disease as related to systemic disease. New evidence forces us to change our methods of diagnosis and treatment of dental infection.

As-yet-uncultivated Oral Phylotypes

As-yet-uncultivated oral phylotypes have been detected in blood samples in episodes of bacteremia following dental procedures³¹, ventilator-associated pneumonia³², sinusitis³³, sputa from cystic fibrosis patients³⁴, and intrauterine infection leading to preterm birth or spontaneous abortion,³⁵ Nordquist and Krutchkoff³⁶, as well as others³⁷, propose evidence that a gross overpopulation of one or many spirochetal species plays an important role in periodontal disease. This finding is based on observation of chronic systemic disease symptoms that are reminiscent of other spirochetal diseases including Lyme disease, relapsing fever, and syphilis.

Traditional culturing techniques of identification of oral microbes in systemic diseases has proved impossible. However, advancements in DNA sequencing (and 16S RNA) analysis have proved invaluable. Siqueira and Rôças, reported 40-60% of the bacteria found in both healthy and diseased oral sites remain to be grown in vitro, phenotypically characterized, and formally named as species³⁸. Therefore, the total number of different oral bacteria species has doubled from about 600 to as many as 1,200.

Like caries and periodontal diseases, the breadth of bacterial diversity in endodontic infections has been substantially expanded by culture-independent molecular methods.³⁹ Sakamoto et al⁴⁰ reported that uncultivated phylotypes accounted for approximately 55% of the taxa found in root canals of teeth with apical periodontitis. In pus aspirates from acute apical abscesses, as-yet uncultivated phylotypes encompassed approximately 24-46% of the taxa found^{41 42}. Rôças⁴³ reports, the “red complex” of bacteria known for their pathology in periodontal disease is also a contributor to apical infection in necrotic teeth. Another demonstrated that the microbiota of symptomatic periapical lesions is predominated by anaerobic bacteria but also contains substantial levels of streptococci, actinomyces, and bacteria not previously identified in the oral cavity⁴⁴.

Traditional culturing techniques are useless when evaluating possible pathogenic microbes in periodontal disease. Alternative methods need to be developed until most oral microbes can be identified and characterized. This will take years and potentially huge monetary expenditures to accomplish. Therefore, older techniques using phase contrast or dark field microscopy technology may be an alternative interim method to bridge the gap in DNA analysis knowledge. There are, of course, services that use DNA data to provide information on imputed levels of specific ‘pathogens’. While valuable, these markers are nowhere near as reliable as we might like to recognize ongoing disease processes or predict with great reliability the likelihood of further breakdown or disease control.

Seek Out and Find Dental Infection

The traditional method for diagnosing periodontal disease comprises using a mechanical periodontal probe with millimeter marks. This probe measures dental pockets around the teeth. When bleeding points occur, these are counted. The two measurements are then used for diagnosis. Considering the gravity of the relationship between dental disease and systemic disease, this method of mechanical diagnosis without evaluating the specific bacteria seems antiquated. Since periodontal disease is an infection, it is paramount to observe the microbes causing the infection directly facilitating identification of specific groups of bacteria, their relative populace related to each other, and their mode and rapidity of movement.

We also need to recognize that the damage seen in periodontal disease is not simply from direct action of the microorganisms against host structures. Actually, it may well be the host's REACTION to these microbes that results in the destruction of host structures. It is the host who generates the matrix metalloproteinases that destroy the integral collagen.⁴⁵ Host levels of inflammatory response must also be modulated to minimize further structural degradation.

When diagnosing necrotic teeth, tradition periapical x-rays leaves much to be desired. In the last ten years or so, new CAT Scan techniques has revolutionized dentistry.

Dental Diagnostic Tools

Diagnosis of periodontal disease: The periodontal microbe milieu is evaluated using the phase contrast microscope electronically enhanced to 5,000X. The microscope is used to calculate imbalances of oral bacteria--specifically oral spirochetes--located in dental plaque. Observation of spirochetal overpopulation signifies a gross imbalance in oral microbes that is easy to recognize. Once detected, diagnosis and treatment options can be formulated. Since there are approximately 60 strains of oral spirochetes, most of which are, as-yet, uncultivated, the microscope is the only available method of identification. Many spirochetes are so minuscule, that they are not visible below the electronic boost to 5,000X. In cases of severe periodontal disease, oral spirochetes can represent most of the visible oral bacteria by number.⁴⁶

Diagnosis of infection located at the apex of necrotic teeth and failed root canal treated

teeth: High definition Cone Beam CAT Scan (CBCT) technology is capable of very high definition using as many as 1000 slices. It can diagnose periapical lesions associated with abscessed teeth, including those lesions located below the root tips of 'successfully' treated root canal teeth. With the advent of this new technology, it has been embarrassingly evident that many lesions are not visible using traditional digital dental radiology. Research has shown that all x-ray-visible lesions associated root canal teeth have an active inflammatory infiltrate^{47 48} and thus a source of microbes for seeding other parts of the body. Moreover, even if these lesions could be sterilized by treatment, without surgically removing the contents, such lesions may well spread inflammatory cytokines and other necrotic waste products throughout the system with further inflammatory or pathogenic effects. Plus, these oxygen starved nooks would serve as rich reservoirs for re-colonization of anaerobic microbes.

Treatment Options

Periodontal disease: Treating periodontal disease requires identification, elimination of pathogenic species, and repopulating the crevicular microbiome. Research has shown that many oral pathogens enter and incubate within gingival sulcus epithelial cells⁴⁹. Therefore, one efficacious method to eliminate these intracellular bacteria along with gingival sulcular microbes is vaporizing them with a micro-beam-tipped CO₂ laser. Then oral hygiene methods should be used to minimize reinfection. The problem remaining after sterilizing the diseased gingival sulcus is rebalancing the microbes. This critical reestablishing of the normal flora is the forefront of research today⁵⁰. Ozone in a variety of forms has also been employed with success, according to personal reports from many practitioners. There do not, however, appear to be many formal research papers published yet on this modality.⁵¹

Endodontic lesions: When extensive decay results in the death of a tooth with a resulting necrotic pulp, endodontic treatment is traditionally performed by dentists. Endodontic treatment is employed to remove and decontaminate the central infected portion of the nerve chamber and main canals of the tooth. However, it is well known by dentists that Root Canal Therapy (RCT) does not effectively remove all the infection associated with the necrotic tooth.

Thousands of dentinal tubules exist within the tooth dentin, each containing a pain sensitive mechanism that innervate the interior of a tooth. Once the tooth dies, these tubules serve as nutrient rich nooks for colonization of bacteria and collect necrotic products that most likely cannot be removed by traditional endodontics. Therefore, extraction is a the most predictable, reliable and effective method of treating necrotic teeth. Also, previously completed endodontic treatment should be evaluated at frequent for radiolucent apical lesions. Any evidence of bone loss at the apex predicts infection remaining associated with the tooth. Therefore, to give the patient the best chance for eliminating oral infection, necrotic teeth with apical lesions must be sacrificed for the better health of the body in general. Aggressive curettage of the bony socket, decontaminating the surrounding area, and grafting is advisable to allow healing with a lowered risk of pathologic infection. If patients insist on saving the tooth, thorough and aggressive endodontic therapy will be necessary. If an apical lesion exists, then a surgical procedure is needed to eliminate the lesion followed by rigorous curettage, decontamination, and grafting. It is highly advisable to give the patient verbal and written informed consent to verify understanding that some of the infection is not removed and can cause problems later.

The dental professional's paradox or the calculus one must employ when making treatment decisions for patients is daunting. It is not 100% certain of a causal relationship between periodontal/endodontic infection and systemic sequelae, but mountains of credible evidence have been collected over many years to convince even the most skeptical critic. For many this is not enough to change years of what is considered the Standard of Care treatment for dental disease. Nonetheless, given this uncertainty, should one then not make any effort to treat the potentially initiating diseases? First, one must also weigh in the negatives, the downsides, the costs of DOING the treatment. Our personal sense is that the downsides are minuscule by comparison to the devastating effects chronic infection and inflammation can potentially have on the entire organism in comparison to the vagaries and difficulties performing the dental treatments.

REFERENCES:

¹ Li X, Kolltveit KM, Tronstad L, Olsen I. Systemic diseases caused by oral infection. Clin Microbiol Rev 2000; 13: 547-58.

Abstract

Recently, it has been recognized that oral infection, especially periodontitis, may affect the course and pathogenesis of a number of systemic diseases, such as cardiovascular disease, bacterial pneumonia, diabetes mellitus, and low birth weight. The purpose of this review is to evaluate the current status of oral infections, especially periodontitis, as a causal factor for systemic diseases. Three mechanisms or pathways linking oral infections to secondary systemic effects have been proposed: (i) metastatic spread of infection from the oral cavity as a result of transient bacteremia, (ii) metastatic injury from the effects of circulating oral microbial toxins, and (iii) metastatic inflammation caused by immunological injury induced by oral microorganisms. Periodontitis as a major oral infection may affect the host's susceptibility to systemic disease in three ways: by shared risk factors; subgingival biofilms acting as reservoirs of gram-negative bacteria; and the periodontium acting as a reservoir of inflammatory mediators. Proposed evidence and mechanisms of the above odontogenic systemic diseases are given.

² Scannapieco FA. Position paper of the American Academy of Periodontology: Periodontal disease as a potential risk factor for systemic diseases. J Periodol 1998;69(7):841-50.

Abstract

This paper on periodontal disease as a potential risk factor for systemic diseases was prepared by the Research, Science and Therapy Committee of The American Academy of Periodontology. It is intended to provide information regarding the role of periodontal disease in systemic diseases, including bacteremia, infective endocarditis, cardiovascular disease and atherosclerosis, prosthetic device infection, diabetes mellitus, respiratory diseases, and adverse pregnancy outcomes.

³ Jodhpur K et al. Strength of evidence linking oral conditions and systemic diseases. Commend Cetin Educ Dent Supply. 2000;(30):12-23.

Abstract

Associations between dental diseases and systemic outcomes are potentially important because of the high occurrence of dental diseases. If this extremely common source of chronic infection (dental disease) leads to an increased morbidity and mortality rate, the public health impact of oral disease on millions of Americans would be substantial. Recent studies demonstrate an association between dental and systemic diseases, including systemic infections, cardiovascular disease, pregnancy outcomes, respiratory diseases, and increased all-cause mortality rate. Because there are several common risk factors for oral and systemic diseases, and limitations in published studies, a careful interpretation is needed. Confounding (shared risk factors for both systemic and dental disease) may explain part of the reported associations. It is also plausible that there may be a causal link. It is likely that if there is a causal link, several pathways and mediators coexist, linking oral and systemic disease. Bacteremia, bacterial endotoxins, cytokines, and other inflammatory mediators could conceivably be playing a direct or indirect role. Missing teeth are a surrogate marker for previous dental infection, and may also lead to altered dietary intake. Hence, diet may be an additional mediator for several of these outcomes. We caution clinicians not to recommend extracting infected teeth, based on the periodontal-systemic disease associations, if the teeth do not warrant extraction otherwise, because loss of teeth and edentulousness are associated with increased risk of systemic diseases. When assessed against causal-defined criteria, the evidence suggests possible causal associations between chronic periodontal disease and tooth loss with cardiovascular disease, bacterial endocarditis, pregnancy outcomes, and all-cause overall mortality. Further studies are needed to show consistency, to corroborate that the associations are independent of common risk factors for both systemic and dental disease, including healthy lifestyle factors, and to evaluate different biological pathways.

⁴ Jodhpur KJ et al. Possible explanations for the tooth loss and cardiovascular disease relationship. Ann Periodontol. 1998;3(1):175-83.

Abstract

Several studies have shown relationships between periodontal disease and cardiovascular disease (CVD). A few studies have also shown that tooth loss may be associated with increased risk of coronary heart

disease and stroke. We have reviewed the relevant literature to assess possible explanations for the reported associations between tooth loss and CVD. In particular, we considered whether the reported association between tooth loss and CVD could be explained by antecedent periodontal disease, antecedent caries, the extraction process, dietary changes following tooth loss, or confounding or bias from other sources. Since access to care and attitudes to health care may influence the decision to extract teeth, as well as cardiovascular disease risk, one needs to be cautious about confounding from behaviorally related factors. Available evidence suggests that further studies are needed to rule out that confounding is a possible explanation for the tooth loss and CVD relationship, that prior periodontal disease may not completely explain the tooth loss-CVD relationship, and that the role of diet needs to be further explored.

⁵ DeStefano F et al. Dental disease and risk of coronary heart disease and mortality. *BMJ*. 1993;306:688-691.

Abstract

OBJECTIVE:

To investigate a reported association between dental disease and risk of coronary heart disease.

SETTING:

National sample of American adults who participated in a health examination survey in the early 1970s.

DESIGN:

Prospective cohort study in which participants underwent a standard dental examination at baseline and were followed up to 1987. Proportional hazards analysis was used to estimate relative risks adjusted for several covariates.

MAIN OUTCOME MEASURES:

Incidence of mortality or admission to hospital because of coronary heart disease; total mortality.

RESULTS:

Among all 9760 subjects included in the analysis those with periodontitis had a 25% increased risk of coronary heart disease relative to those with minimal periodontal disease. Poor oral hygiene, determined by the extent of dental debris and calculus, was also associated with an increased incidence of coronary heart disease. In men younger than 50 years at baseline periodontal disease was a stronger risk factor for coronary heart disease; men with periodontitis had a relative risk of 1.72. Both periodontal disease and poor oral hygiene showed stronger associations with total mortality than with coronary heart disease.

CONCLUSION:

Dental disease is associated with an increased risk of coronary heart disease, particularly in young men. Whether this is a causal association is unclear. Dental health may be a more general indicator of personal hygiene and possibly health care practices.

⁶ Loesche WJ. et al. Periodontal disease as a risk factor for heart disease. *Compend Contin Educ Dent*.1994;25(8):976-992.

Abstract

Many individuals with cardiovascular disease appear from epidemiologic studies to have either periodontal disease or to be edentulous. A Finnish group has provided evidence that after conventional risk factors for stroke and heart attacks have been accounted for, there still remains a significant relationship between dental disease and cardiovascular disease. A preliminary analysis of our own investigation of the interrelationship of medical and dental health shows that individuals with a high dental morbidity (ie, edentulous or with many missing teeth) have a high prevalence of coronary heart disease and stroke.

⁷ Loesche WJ et al. Periodontal disease: link to cardiovascular disease. *Compend Contin Educ Dent*. 2000;21(6):463-6, 468, 470 passim.

Abstract

Poor oral hygiene that leads to dental infections could contribute to adverse medical outcomes such as cardiovascular disease. Twelve studies of varying degrees of design rigor have associated dental conditions, such as periodontal disease, missing teeth, and edentulousness, with either coronary heart disease or a cerebral vascular accident. Six of the studies were longitudinal so that the demonstration of the oral health parameters as significant predictors of the cardiovascular event would elevate the dental parameter to the status of a risk factor. Because dental diseases (especially periodontal disease) are

treatable, the dental component is a modifiable risk factor; therefore, maintaining good oral health should receive the highest priority for a healthy life.

⁸ Behle JH, Papapanou PN. Periodontal infections and atherosclerotic vascular disease: an update. *Int Dent J.* 2006;56(4 Suppl 1):256-62.

Abstract

The role of periodontal infections as a putative risk factor for atherosclerotic vascular disease (ASVD) has been reported in the literature over the past decade. This review provides insights into biologically plausible pathways that can potentially mediate such an association, and discusses recent findings from epidemiological studies and intervention trials. Accumulating epidemiological evidence suggests that clinical, microbiological and serological markers of periodontal infection are associated with subclinical and manifest ASVD. Early evidence from intervention studies suggests that the control of periodontal infections may result in improved levels of markers of systemic inflammation and measures of endothelial dysfunction.

⁹ Geerts SO, et al. Further evidence of the association between periodontal conditions and coronary artery disease. *J Periodontol.* 2004;75(9):1274-80.

Abstract

BACKGROUND:

There is increasing evidence that chronic infections, such as periodontal diseases, could play a role in the initiation and development of coronary artery disease (CAD). The present study was intended to test for a possible association between presence and severity of periodontitis and coronary artery disease in a Belgian population.

METHODS:

A total of 108 CAD patients (mean age 59.2 +/- 11 years) and 62 presumably healthy controls (mean age 57.7 +/- 9 years) were enrolled in the study. Probing depth, periodontal pocket bleeding index (PPBI), plaque index, furcation involvements, and tooth mobility were evaluated to compare periodontal health in both groups. The subjects were also ranked according to a novel index of periodontitis severity, the periodontal index for risk of infectiousness (PIRI), aimed at quantifying the risk of release of proinflammatory mediators from the periodontal sites.

RESULTS:

Periodontitis was significantly more frequent in CAD patients than in controls (CAD patients: 91%; controls: 66%). The mean number of pockets was 18 +/- 17.1 in cardiac patients versus 7.6 +/- 12.7 in controls ($P < 0.0001$), despite the fact that the mean number of missing teeth was significantly greater in cases than in controls (14 +/- 7.1 versus 9 +/- 5.2; $P < 0.0001$). Furthermore, proportions of mobile teeth, bleeding sites, periodontal pockets, and involved furcations were significantly higher in CAD patients than in controls. In addition, the extent of the periodontal disease present was also greater in cases than in controls. A logistic model, adjusted for known cardiovascular risk factors, showed a strong association between CAD and periodontitis (odds ratio [OR] = 6.5). Moreover, there was a significant dose-response relationship between increasing scores of the periodontal risk of infectiousness and the presence of CAD (adjusted OR = 1.3 per PIRI unit).

CONCLUSION:

In the present study, periodontitis was revealed to be a significant risk factor for CAD after adjusting for other confounding factors, with the level of association increasing with the individual extent of the periodontal lesions.

¹⁰ Haraszthy VI, Zambon JJ, Trevisan M, Zeid M, Genco RJ. Identification of periodontal pathogens in atheromatous plaques. *J Periodontol* 2000; 71: 155460.

Abstract

BACKGROUND:

Recent studies suggest that chronic infections including those associated with periodontitis increase the risk for coronary vascular disease (CVD) and stroke. We hypothesize that oral microorganisms including periodontal bacterial pathogens enter the blood stream during transient bacteremias where they may play a role in the development and progression of atherosclerosis leading to CVD.

METHODS:

To test this hypothesis, 50 human specimens obtained during carotid endarterectomy were examined for the presence of *Chlamydia pneumoniae*, human cytomegalovirus, and bacterial 16S ribosomal RNA using specific oligonucleotide primers in polymerase chain reaction (PCR) assays. Approximately 100 ng of chromosomal DNA was extracted from each specimen and then amplified using standard conditions (30 cycles of 30 seconds at 95 degrees C, 30 seconds at 55 degrees C, and 30 seconds at 72 degrees C). Bacterial 16S rDNA was amplified using 2 synthetic oligonucleotide primers specific for eubacteria. The PCR product generated with the eubacterial primers was transferred to a charged nylon membrane and probed with digoxigenin-labeled synthetic oligonucleotides specific for *Actinobacillus actinomycetemcomitans*, *Bacteroides forsythus*, *Porphyromonas gingivalis*, and *Prevotella intermedia*.

RESULTS:

Eighty percent of the 50 endarterectomy specimens were positive in 1 or more of the PCR assays. Thirty-eight percent were positive for HCMV and 18% percent were positive for *C. pneumoniae*. PCR assays for bacterial 16S rDNA also indicated the presence of bacteria in 72% of the surgical specimens. Subsequent hybridization of the bacterial 16S rDNA positive specimens with species-specific oligonucleotide probes revealed that 44% of the 50 atheromas were positive for at least one of the target periodontal pathogens. Thirty percent of the surgical specimens were positive for *B. forsythus*, 26% were positive for *P. gingivalis*, 18% were positive for *A. actinomycetemcomitans*, and 14% were positive for *P. intermedia*. In the surgical specimens positive for periodontal pathogens, more than 1 species was most often detected. Thirteen (59%) of the 22 periodontal pathogen-positive surgical specimens were positive for 2 or more of the target species.

CONCLUSIONS:

Periodontal pathogens are present in atherosclerotic plaques where, like other infectious microorganisms such as *C. pneumoniae*, they may play a role in the development and progression of atherosclerosis leading to coronary vascular disease and other clinical sequelae.

¹¹Bale BF1, Doneen AL1, Vigerust DJ2. High-risk periodontal pathogens contribute to the pathogenesis of atherosclerosis. *Postgrad Med J.* 2017 Apr;93(1098):215-220.

Abstract

*Periodontal disease (PD) is generated by microorganisms. These microbes can enter the general circulation causing a bacteraemia. The result can be adverse systemic effects, which could promote conditions such as cardiovascular disease. Level A evidence supports that PD is independently associated with arterial disease. PD is a common chronic condition affecting the majority of Americans 30 years of age and older. Atherosclerosis remains the largest cause of death and disability. Studies indicate that the adverse cardiovascular effects from PD are due to a few putative or high-risk bacteria: *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola* or *Fusobacterium nucleatum* There are three accepted essential elements in the pathogenesis of atherosclerosis: lipoprotein serum concentration, endothelial permeability and binding of lipoproteins in the arterial intima. There is scientific evidence that PD caused by the high-risk pathogens can influence the pathogenesis triad in an adverse manner. With this appreciation, it is reasonable to state PD, due to high-risk pathogens, is a contributory cause of atherosclerosis. Distinguishing this type of PD as causal provides a significant opportunity to reduce arterial disease.*

¹² Miklossy J, McGeer PL. Common mechanisms involved in Alzheimer's disease and type 2 diabetes: a key role of chronic bacterial infection and inflammation. *Aging (Albany NY).* 2016 Apr;8(4):575-88.

Abstract

*Strong epidemiologic evidence and common molecular mechanisms support an association between Alzheimer's disease (AD) and type 2-diabetes. Local inflammation and amyloidosis occur in both diseases and are associated with periodontitis and various infectious agents. This article reviews the evidence for the presence of local inflammation and bacteria in type 2 diabetes and discusses host pathogen interactions in chronic inflammatory disorders. *Chlamydomypha pneumoniae*, *Helicobacter pylori* and spirochetes are demonstrated in association with dementia and brain lesions in AD and islet lesions in type 2 diabetes. The presence of pathogens in host tissues activates immune responses through Toll-like*

receptor signaling pathways. Evasion of pathogens from complement-mediated attack results in persistent infection, inflammation and amyloidosis. Amyloid beta and the pancreatic amyloid called amylin bind to lipid bilayers and produce Ca(2+) influx and bacteriolysis. Similarly to AD, accumulation of amylin deposits in type 2 diabetes may result from an innate immune response to chronic bacterial infections, which are known to be associated with amyloidosis. Further research based on an infectious origin of both AD and type 2 diabetes may lead to novel treatment strategies.

¹³ Chapple IL, Genco R; working group 2 of the joint EFP/AAP workshop. Diabetes and periodontal diseases: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. J Periodontol. 2013 Apr;84(4 Suppl):S106-12.

Abstract

BACKGROUND:

Diabetes and periodontitis are complex chronic diseases with an established bidirectional relationship. There is long-established evidence that hyperglycaemia in diabetes is associated with adverse periodontal outcomes. However, given the ubiquity of periodontal diseases and the emerging global diabetes epidemic, the complications of which contribute to significant morbidity and premature mortality, it is timely to review the role of periodontitis in diabetes.

AIMS:

To report the epidemiological evidence from cross-sectional, prospective and intervention studies for the impact of periodontal disease on diabetes incidence, control and complications and to identify potential underpinning mechanisms.

EPIDEMIOLOGY:

Over the last 20 years, consistent and robust evidence has emerged that severe periodontitis adversely affects glycaemic control in diabetes and glycaemia in non-diabetes subjects. In diabetes patients, there is a direct and dose-dependent relationship between periodontitis severity and diabetes complications. Emerging evidence supports an increased risk for diabetes onset in patients with severe periodontitis. Biological mechanisms: Type 2 diabetes is preceded by systemic inflammation, leading to reduced pancreatic b-cell function, apoptosis and insulin resistance. Increasing evidence supports elevated systemic inflammation (acute-phase and oxidative stress biomarkers) resulting from the entry of periodontal organisms and their virulence factors into the circulation, providing biological plausibility for the effects of periodontitis on diabetes. AGE (Advanced Glycation Endproducts)-RAGE (Receptor for AGEs) interactions and oxidative-stress-mediated pathways provide plausible mechanistic links in the diabetes to periodontitis direction.

INTERVENTIONS:

Randomized controlled trials (RCTs) consistently demonstrate that mechanical periodontal therapy associates with approximately a 0.4% reduction in HbA1C at 3 months, a clinical impact equivalent to adding a second drug to a pharmacological regime for diabetes. RCTs are needed with larger numbers of subjects and longer term follow-up, and if results are substantiated, adjunctive periodontal therapies subsequently need to be evaluated. There is no current evidence to support adjunctive use of antimicrobials for periodontal management of diabetes patients.

GUIDELINES:

Given the current evidence, it is timely to provide guidelines for periodontal care in diabetes patients for medical and dental professionals and recommendations for patients/the public.

¹⁴ Singhrao SK, Harding A, Poole S, Kesavalu L, Crean S. Porphyromonas gingivalis Periodontal Infection and Its Putative Links with Alzheimer's Disease. Mediators Inflamm. 2015;2015:137357. doi: 10.1155/2015/137357. Epub 2015 Apr 30.

Abstract

Periodontal disease (PD) and Alzheimer's disease (AD) are inflammatory conditions affecting the global adult population. In the pathogenesis of PD, subgingival complex bacterial biofilm induces inflammation that leads to connective tissue degradation and alveolar bone resorption around the teeth. In health, junctional epithelium seals the gingiva to the tooth enamel, thus preventing bacteria from entering the gingivae. Chronic PD involves major pathogens (Porphyromonas gingivalis, Treponema denticola, and

Tannerella forsythia) which have an immune armory that can circumvent host's immune surveillance to create and maintain an inflammatory mediator rich and toxic environment to grow and survive. The neurodegenerative condition, AD, is characterized by poor memory and specific hallmark proteins; periodontal pathogens are increasingly being linked with this dementing condition. It is therefore becoming important to understand associations of periodontitis with relevance to late-onset AD. The aim of this review is to discuss the relevance of finding the keystone periodontal pathogen *P. gingivalis* in AD brains and its plausible contribution to the etiological hypothesis of this dementing condition.

- ¹⁵ Wu S et al. Cognitive function and oral health among community-dwelling older adults. *J Gerontol A Biol Sci Med Sci.* 2008 May;63(5):495-500.

Abstract

BACKGROUND:

Both oral health problems and cognitive impairment are relatively common among older adults. Poorer oral health appears to contribute to a decline in quality of life and to be related to various medical conditions. Little is known about the relationship of cognitive function to oral health among community-dwelling older adults.

METHODS:

The sample included 1984 dentate community-dwelling older adults 60 years old or older from the National Health and Nutrition Examination Survey (NHANES, 1999-2002) who completed both the study cognitive measure and dental examination. Weighted descriptive and multivariate regression analyses were performed.

RESULTS:

Multivariate analyses showed that cognitive function was associated with oral health. Individuals with lower cognitive scores had a higher number of decayed and missing teeth and a higher proportion of periodontitis sites. The predicted number of decayed teeth increased by 0.01 with each 1-point decrease in the Digit Symbol Substitution Test score; the number of missing teeth increased by 0.02; and the percentage of sites with periodontal disease increased by 0.02. In addition, individuals' sociodemographic characteristics, health behavior, and regular dental checkups were significantly associated with oral health.

CONCLUSIONS:

This study suggests that community-dwelling elders with lower cognitive function scores have greater deterioration of oral health. This study provides a preliminary knowledge base for the development of early intervention strategies to address oral health problems among older adults.

- ¹⁶ Avlund K. Tooth loss and caries prevalence in very old Swedish people: the relationship to cognitive and functional ability. *Gerodontology.* 2004;21(1):17-26.

Abstract

OBJECTIVE:

To analyse whether cognitive function and functional ability are related to oral health among community-dwelling older people over the age of 80 years.

BACKGROUND:

This cross-sectional study is based on the Kungsholmen Elders Oral Health Survey (KEOHS). The study included oral examinations carried out in two local clinics by standardised examiners and interviews using structured questionnaires.

MATERIALS AND METHODS:

Altogether 159 individuals were included in this study. Coronal caries and root caries were assessed using the National Institute of Dental and Craniofacial Research (NIDCR) diagnostic criteria. Cognitive function was assessed by the Mini-Mental State Examination (MMSE) index and functional ability was assessed by a global measure of self-reported changes.

RESULTS:

Older adults with a low MMSE score (< or = 23) tended to have a higher risk of coronal caries than those with higher scores. Participants with mild cognitive decline (MMSE = 24-26) and with a decrease in functional ability had a significantly higher risk of root caries. These associations changed little when

adjusted by the covariates. In addition, people with a low MMSE (0-23) had a four times higher risk of not using dental services regularly. This result was unchanged after adjusting for the variables studied.

CONCLUSIONS:

This study revealed associations between the cognitive and functional status of the individual and aspects of oral health, that may contribute to a deeper understanding of the background of oral health status in older adults.

- ¹⁷ Stewart R. Oral health and cognitive function in the Third national health and Nutrition Examination Survey (NHANES III). *Psychosom Med.* 2008 Oct;70(8)936-41.

Abstract

OBJECTIVES:

To investigate the association between oral health and cognitive function in early-, mid-, and late-adult life.

METHODS:

A secondary analysis was carried out of a large, well-characterized community sample (NHANES III). Analyzed variables included three measures of oral health (gingival bleeding, loss of periodontal attachment, loss of teeth) and three measures of cognitive function: the Symbol Digit Substitution Test (SDST), the Serial Digit Learning Test (SDLT) (both in 5138 participants aged 20-59 years), and a Story Recall test (in 1555 participants aged ≥ 70 years). Other covariates in linear regression models included age, gender, ethnicity, education and poverty, and cardiovascular risk factors.

RESULTS:

Worse scores on all three measures of oral health status were significantly associated with poorer performance on all three measures of cognitive function after adjustment for age. Education was an important confounding factor. However, after full adjustment for all other covariates, gingival bleeding (%) and loss of periodontal attachment (%) remained associated with relative impairment on SDST score (B coefficients both = 0.003), and gingival bleeding was associated with relative impairment on SDLT (B = 0.017). No effect modification by age was observed.

CONCLUSIONS:

Poor oral health is associated with worse cognitive function throughout adult life. This may, in part, be accounted for by early life education and social status. However, the possibility of direct causal pathways requires further investigation.

- ¹⁸ Wu B, Plassman BL, Liang J, and Wei L. Cognitive Function and Dental Care Utilization Among Community-Dwelling Older Adults. *Am J Public Health.* 2007 December; 97(12): 2216–2221.

Abstract

Objectives. We sought to investigate the relationship between varying levels of cognitive function and dental care utilization.

Methods. Using data obtained from the National Health and Nutrition Examination Survey (1999–2002), we performed weighted descriptive and multivariate logistic regression analyses on 1984 individuals with at least 1 tooth and who were 60 years and older.

Results. Multivariate analyses suggested that level of cognitive function was associated with dental care utilization. At a higher level of cognitive functioning, individuals were more likely to have had more frequent dental visits. In addition, a higher level of socioeconomic status, healthy lifestyle, and worse self-rated oral health–related symptoms were more likely to indicate a higher frequency of dental care utilization. By contrast, poorer oral health status as determined by clinical examinations was negatively associated with frequency of dental visits.

Conclusions. The results suggest that community-dwelling older adults with low cognitive function are at risk for less frequent use of dental care. Oral health serves as a mediating factor between cognitive function and dental care utilization. There is a great need to improve oral health awareness and education among older adults, caregivers, and health care professionals.

- ¹⁹ Okamoto N, Morikawa M, Okamoto K, Habu N, Iwamoto J, Tomioka K, et al. Relationship of tooth loss to mild memory impairment and cognitive impairment: findings from the Fujiwara-kyo study. *Behavioral and Brain Functions* 2010, 6:77.

Abstract

BACKGROUND:

This cross-sectional study investigated the relationship between the number of remaining teeth to mild memory impairment (MMI), which is a preclinical stage of dementia, and to cognitive impairment.

METHODS:

The subjects were aged 65 years or older and were grouped according to their score for the Mini-Mental State Examination (MMSE), the three-word delayed recall test in the MMSE, and the Geriatric Depression Scale into the control group (n = 3,696), the MMI group (n = 121), and the low MMSE score (23 or lower) group (n = 214). We collected data on the number of remaining teeth, the length of the edentulous period, health-related lifestyle, medical history, blood pressure, height, and body weight. Fasting venous blood samples were also obtained.

RESULTS:

Multiple logistic regression analysis, adjusted for depressive symptoms, age, sex, length of education, and other explanatory variables, revealed that the odds ratios of 0-10 remaining teeth to 22-32 remaining teeth were 1.679 (95% CI 1.073-2.627) for MMI and 2.177 (95% CI 1.510-3.140) for a low MMSE score. A significant relationship was also found between the length of the edentulous period and the risk of a low MMSE score (odds ratio 3.102, 95% CI 1.432-6.720) (15 years or more/less than 15 years).

CONCLUSIONS:

Our findings suggest that tooth loss is associated with cognitive function.

²⁰ Miklossy J. Bacterial Amyloid and DNA are Important Constituents of Senile Plaques: Further Evidence of the Spirochetal and Biofilm Nature of Senile Plaques. *J Alzheimer's Dis.* 2016 Jun 13;53(4):1459-73.

Abstract

It has long been known that spirochetes form clumps or micro colonies in vitro and in vivo. Cortical spirochetal colonies in syphilitic dementia were considered as reproductive centers for spirochetes. Historic and recent data demonstrate that senile plaques in Alzheimer's disease (AD) are made up by spirochetes. Spirochetes are able to form biofilm in vitro. Senile plaques are also reported to contain elements of biofilm constituents. We expected that A β PP and A β (the main components of senile plaques) also occur in pure spirochetal biofilms, and bacterial DNA (an important component of biofilm) is also present in senile plaques. Histochemical, immunohistochemical, and in situ hybridization techniques and the TUNEL assay were used to answer these questions. The results obtained demonstrate that A β and DNA, including spirochete-specific DNA, are key components of both pure spirochetal biofilms and senile plaques in AD and confirm the biofilm nature of senile plaques. These results validate previous observations that A β PP and/or an A β PP-like amyloidogenic protein are an integral part of spirochetes, and indicate that bacterial and host derived A β are both constituents of senile plaques. DNA fragmentation in senile plaques further confirms their bacterial nature and provides biochemical evidence for spirochetal cell death. Spirochetes evade host defenses, locate intracellularly, form more resistant atypical forms and notably biofilms, which contribute to sustain chronic infection and inflammation and explain the slowly progressive course of dementia in AD. To consider co-infecting microorganisms is equally important, as multi-species biofilms result in a higher resistance to treatments and a more severe dementia.

²¹ Itzhaki RF, Lathe R, Balin BJ, Ball MJ, Bearer EL, Braak H, Bullido MJ, Carter C, Clerici M, Cosby SL, Del Tredici K, Field H, Fulop T, Grassi C, Griffin WS, Haas J, Hudson AP, Kamer AR, Kell DB, Licastro F, Letenneur L, Lövheim H, Mancuso R, Miklossy J, Otth C, Palamara AT, Perry G, Preston C, Pretorius E, Strandberg T, Tabet N, Taylor-Robinson SD, Whittum-Hudson JA. *Microbes and Alzheimer's Disease.*

Abstract

We are researchers and clinicians working on Alzheimer's disease (AD) or related topics, and we write to express our concern that one particular aspect of the disease has been neglected, even though treatment based on it might slow or arrest AD progression. We refer to the many studies, mainly on humans, implicating specific microbes in the elderly brain, notably herpes simplex virus type 1 (HSV1), Chlamydia pneumoniae, and several types of spirochaete, in the etiology of AD [1-4]. Fungal infection of AD brain [5, 6] has also been described, as well as abnormal microbiota in AD patient blood [7]. The first observations

of HSV1 in AD brain were reported almost three decades ago [8]. The ever-increasing number of these studies (now about 100 on HSV1 alone) warrants re-evaluation of the infection and AD concept.

²² Riviere GR. Molecular and immunological evidence of oral treponema in the human brain and their association with Alzheimer's disease. *Oral Micro Imm* 2002;17:113-118.

Abstract

The purpose of this investigation was to use molecular and immunological techniques to determine whether oral Treponema infected the human brain. Pieces of frontal lobe cortex from 34 subjects were analyzed with species-specific PCR and monoclonal antibodies. PCR detected Treponema in 14/16 Alzheimer's disease (AD) and 4/18 non-AD donors (P < 0.001), and AD specimens had more Treponema species than controls (P < 0.001). PCR also detected Treponema in trigeminal ganglia from three AD and two control donors. Cortex from 15/16 AD subjects and 6/18 controls contained Treponema pectinovorum and/or Treponema socranskii species-specific antigens (P < 0.01). T. pectinovorum and/or T. socranskii antigens were also found in trigeminal ganglia and pons from four embalmed cadavers, and 2/4 cadavers also had Treponema in the hippocampus. These findings suggest that oral Treponema may infect the brain via branches of the trigeminal nerve.

²³ MacDonald A. Review of Infectious Borrelia species Chronic Brain Infections and the Development of Alzheimer's Disease. 2015 <https://vimeo.com/145745120>.

Abstract

Very recent research has proven that Amyloid plaque—the hallmark of Alzheimer's disease—is an active biofilm of spirochetes. 18 MacDonald presented 100 cases of brain tissue harvested from deceased Alzheimer's patients. In this 37-minute video, Borrelia Chronic Brain Infections and development of Alzheimer's Disease, MacDonald used specific DNA marker probes to identify Lyme disease spirochetes, if present. The results were remarkable. The research tools used by MacDonald include:

1. 5 cases of fresh autopsy Alzheimer's brain tissue which revealed positive recovery of Borrelia burgdorferi (Lyme) spirochetes.

2. Use of Borrelia burgdorferi Specific DNA probes to image single Borrelia spirochetes. Plus, this probe identified biofilm communities of Borrelia spirochetes in Alzheimer's amyloid plaques.

MacDonald, for the very first time, demonstrated the role of granular forms of Borrelia, as viable, virulent pathogens, distinct from the spiral Borrelia corkscrew shaped forms. He identified round body ("spore-like" or circular) forms of spirochetes in the evolution of Alzheimer's Disease. All these forms live within active biofilm communities previously defined as undefined dead-brown appearing lesions called amyloid plaques. This seminal research demonstrated all the pleomorphic forms of spirochetes. Some of these forms were identified, but not universally recognized, for over one hundred years.

²⁴ Li X, Tronstad L, Olsen I. Brain abscesses caused by oral infection. *Endod Dent Traumatol* 1999; 15: 95101.

Abstract

Brain abscesses are rare but can be life-threatening infections. Recent progress in microbiological classification and identification has indicated that they are sometimes caused by oral infection and dental treatment. It has been postulated that oral microorganisms may enter the cranium by several pathways: 1) by direct extension, 2) by hematogenous spread, 3) by local lymphatics, and 4) indirectly, by extraoral odontogenic infection. In the direct extension, oral infections spread along the fascial planes. Hematogenous spreading occurs along the facial, angular, ophthalmic, or other veins which lack valves, through the cavernous sinus and into the cranium. Another hematogenous pathway is through the general circulation. Oral bacteria may cause systemic infections, e.g., endocarditis, and then indirectly initiate brain abscess. Microbiota, complications, and the prevention and management of odontogenic brain abscesses are also discussed in this review.

²⁵ Sun J, Zhou M, Salazar CR, Hays R, Bedi S, Chen Y, Li Y. Chronic Periodontal Disease, Periodontal Pathogen Colonization, and an Increased Risk of Precancerous Gastric Lesions. *J Periodontol*. 2017 Jul 3:1-19.

Abstract

BACKGROUND:

The present study assessed the association between periodontal pathogen colonization and the potential risk of developing precancerous lesions of gastric cancer (PLGC) in a clinical setting.

METHODS:

The present study included 35 newly diagnosed patients with PLGC and 70 age-matched individuals without PLGC. A full-mouth intra-oral examination was performed to assess the periodontal conditions. Stimulated whole saliva and pooled plaque samples were collected to evaluate colonization by *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia* and *Actinobacillus actinomycetemcomitans* and to characterize the oral microbial diversity in the saliva and dental plaque.

RESULTS:

Compared with the control group, the patients with PLGC experienced a higher prevalence of bleeding on probing (BOP; 31.5% vs. 22.4%, $P < 0.05$), higher levels of *T. denticola* ($P < 0.01$) and *A. actinomycetemcomitans* ($P < 0.01$), and less bacterial diversity in their saliva ($P < 0.01$). The final multivariate logistic regression model comprising all key socio-demographic characteristics, oral health behavioral factors and periodontal assessments revealed that elevated colonization with periodontal pathogens, specifically *T. forsythia*, *T. denticola*, and *A. actinomycetemcomitans*, decreased bacterial diversity in the dental plaque, and not flossing teeth regularly were significant predictors of an increased risk of PLGC ($P = 0.022$).

CONCLUSION:

The findings of the present study provide new evidence suggesting that periodontal pathogen burdens and bacterial diversity in the oral cavity are important factors contributing to a potential increased risk of developing precancerous gastric lesions.

²⁶ Estemalik J1, Demko C2, Bissada NF1, Joshi N1, Bodner D3, Shankar E3, Gupta S3. Simultaneous Detection of Oral Pathogens in Subgingival Plaque and Prostatic Fluid of Men with Periodontal and Prostatic Diseases. J Periodontol. 2017 May 26:1-11.

Abstract

BACKGROUND:

Chronic prostatitis (CP) and benign prostatic hyperplasia (BPH) are complex inflammatory conditions for which the etiological determinants are still poorly defined. Periodontitis is caused by subgingival colonizing bacteria in the oral cavity. The causal effect of periodontal disease on prostatic inflammation has not been established. The purpose of this study is to isolate oral pathogens from expressed prostatic secretions of patients with periodontal disease and chronic prostatitis or benign prostatic hyperplasia.

METHODS:

Twenty-four men diagnosed with CP/BPH participated in the study. A complete periodontal examination consisting of probing depth, bleeding on probing, tooth mobility, gingival index and plaque index was performed on men and prostatic secretion was collected for the study. Dental plaque and prostatic secretion samples were used for analysis of bacterial DNA for *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola* and *Escherichia coli* using RT-PCR.

RESULTS:

Six patients were diagnosed with severe, 7 with moderate, and 4 with mild chronic periodontitis. Seventeen out of 24 (70.8%) of the prostatic secretion samples showed one or more of the studied oral pathogens. Nine out of the 10 BPH and 8 out of the 14 patients with prostatitis had at least one oral pathogen in their prostatic secretions. *P. gingivalis* was found in both prostatic secretion and plaque samples in 6 out of 17 (35.3%) patients, *T. denticola* in both samples, 7 out of 15 (46.7%) and *E. coli* in both samples 3 out of 15 (20%) patients. *P. intermedia* was detected in all dental plaque samples but not in the prostatic secretion.

CONCLUSIONS:

An association between chronic inflammatory prostate and periodontal diseases has been demonstrated by the presence of similar bacterial DNA in both the prostatic secretion and subgingival dental plaque from same individual.

²⁷ Abnet, et al. Tooth loss is associated with increased risk of total death and death from upper gastrointestinal cancer, heart disease and stroke. *Int J Epidemiol.* 2005 Apr;34(2):475-6.

Abstract

BACKGROUND:

Tooth loss has previously been associated with a higher risk of cancer, heart disease, and stroke, but the role of confounding by smoking remains an issue.

METHODS:

We conducted a cohort study including 29,584 healthy, rural Chinese adults who were participants in a chemoprevention trial from 1986 through 1991 and who have been followed-up through 2001. We categorized tooth loss for each subject as less than or equal to or greater than the median number of teeth lost for other subjects of the same age at baseline. Mortality outcomes were categorized as follows: total death (n = 9362), upper gastrointestinal (GI) cancer death (n = 2625), other cancer death (n = 514), heart disease death (n = 1932), and fatal stroke (n = 2866).

RESULTS:

Individuals with greater than the age-specific median number of teeth lost had statistically significant 13% increased risk of total death [95% confidence interval (CI) 9-18%], 35% increased risk of upper GI cancer death (95% CI 14-59%), 28% increased risk of heart disease death (95% CI 17-40%), and 12% increased risk of stroke death (95% CI 2-23%), but no significantly increased risk of death from cancer at other sites. These elevated risks were present in male smokers, male non-smokers, and females, nearly all never-smokers.

CONCLUSIONS:

In this Asian population, tooth loss significantly increased the risk of total death and death from upper GI cancer, heart disease, and stroke. These associations were not limited to tobacco smokers.

²⁸ Hiraki et al. Teeth loss and risk of cancer at 14 sites in Japanese. *Cancer Epidemiol Biomarkers Prev.* 2008 May;17(5):1222-7.

Abstract

BACKGROUND:

Tooth loss has been associated with a higher risk of several types of cancer. To clarify the significance of tooth loss to the risk of 14 common cancers, we conducted a large-scale, case-control study based on the Hospital-based Epidemiologic Research Program at Aichi Cancer Center.

METHODS:

A total of 5,240 cancer subjects and 10,480 age- and sex-matched noncancer controls were recruited. Patients with 14 types of cancer newly diagnosed from 2000 to 2005 were eligible as case subjects, and new outpatients without cancer in the same time period were eligible as controls. Tooth loss was categorized into four groups: group 1, number of remaining teeth, ≥ 21 ; group 2, 9 to 20; group 3, 1 to 8; and group 4, 0. The effect of tooth loss was assessed as odds ratios (OR) with 95% confidence intervals (95% CI) calculated with conditional logistic regression models, with adjustment for potential confounders.

RESULTS:

A decreased number of remaining teeth was associated with increased OR of head and neck (OR, 1.68; 95% CI, 0.88-1.93; P trend = 0.055), esophageal (OR, 2.36; 95% CI, 1.17-4.75; P trend = 0.002), and lung (OR, 1.54; 95% CI, 1.05-2.27; P trend = 0.027) cancers.

CONCLUSIONS:

We showed a significant positive association between tooth loss and the risk of head and neck, esophageal, and lung cancers after adjustment for potential confounding factors. The findings indicate that preventive efforts aimed at the preservation of teeth may decrease the risk of these cancers.

²⁹ Tezal M. Chronic periodontitis and the risk of tongue cancer. *Arch. Otolaryn Head and Neck Surgery.* 2007;133(5):450-454.

Abstract

OBJECTIVE:

To assess the association between the history of chronic periodontitis and the risk of tongue cancer.

DESIGN:

Case-control study using preexisting data from patients admitted between June 15, 1999, and November 17, 2005.

SETTING:

Department of Dentistry and Maxillofacial Prosthetics at Roswell Park Cancer Institute (RPCI), Buffalo, NY.

PATIENTS:

The cases comprised 51 non-Hispanic white men newly diagnosed as having primary squamous cell carcinoma of the tongue, and the controls, 54 non-Hispanic white men evaluated during the same period but with negative results for malignancy. Children (aged <21 years), edentulous or immunocompromised patients, and those with history of any cancer were excluded. History of periodontitis was assessed by alveolar bone loss measured from panoramic radiographs by 1 examiner blind to cancer status.

MAIN OUTCOME MEASURE:

Incidence of tongue cancer obtained from the RPCI Tumor Registry.

RESULTS:

After adjusting for the effects of age at diagnosis, smoking status, and number of teeth, each millimeter of alveolar bone loss was associated with a 5.23-fold increase in the risk of tongue cancer (odds ratio, 5.23; 95% confidence interval, 2.64-10.35).

CONCLUSIONS:

This study suggests an association between chronic periodontitis and the risk of tongue cancer in men, independent of smoking status, age, race, ethnicity, and number of teeth. This association needs to be confirmed by larger studies using quantitative assessment of lifetime tobacco exposure. If this association is confirmed, it has a potential impact on understanding the etiology of oral cancer as well as on its prevention and control.

³⁰ Nordquist WD and Krutchkoff DJ, *The Silent Saboteurs* 2010. Bio Med Publishing Group. Lake Tahoe, CA.

Abstract

Compares the long-term symptoms of periodontal disease—oral spirochetosis—with syphilis and Lyme disease. Identifies oral spirochetes as a possible instigator of systemic disease.

³¹ Bahrani-Mougeot FK, Paster BJ, Coleman S, Ashar J, Barbuto S, Lockhart PB. Diverse and novel oral bacterial species in blood following dental procedures. *J Clin Microbiol* 2008; 46:2129-32.

Abstract

We identified oral bacterial species in blood cultures following single-tooth extraction and tooth brushing. Sequence analysis of 16S rRNA genes identified 98 different bacterial species recovered from 151 bacteremic subjects. Of interest, 48 of the isolates represented 19 novel species of Prevotella, Fusobacterium, Streptococcus, Actinomyces, Capnocytophaga, Selenomonas, and Veillonella.

³² Bahrani-Mougeot FK, Paster BJ, Coleman S, Barbuto S, Brennan MT, Noll J, et al. Molecular analysis of oral and respiratory bacterial species associated with ventilator-associated pneumonia. *J Clin Microbiol* 2007; 45: 1588-93.

Abstract

Trauma intensive care unit (TICU) patients requiring mechanical respiratory support frequently develop ventilator-associated pneumonia (VAP). Oral and oropharyngeal bacteria are believed to be responsible for many cases of VAP, but definitive evidence of this relationship is lacking. Earlier studies used conventional culture-based methods for identification of bacterial pathogens, but these methods are insufficient, as some bacteria may be uncultivable or difficult to grow. The purpose of this study was to use a culture-independent molecular approach to analyze and compare the bacterial species colonizing the oral cavity and the lungs of TICU patients who developed VAP. Bacterial samples were acquired from the dorsal tongue and bronchoalveolar lavage fluid of 16 patients. Bacterial DNA was extracted, and the 16S rRNA genes were PCR amplified, cloned into Escherichia coli, and sequenced. The sequencing data revealed the following: (i) a wide diversity of bacterial species in both the oral and pulmonary sites, some of them novel; (ii) known and putative respiratory pathogens colonizing both the oral cavity and lungs of 14 patients; and (iii) a number of bacterial pathogens (e.g., Dialister pneumosintes, Haemophilus segnis, Gemella morbillorum, and Pseudomonas fluorescens) in lung samples that had not been reported previously at this site when culture-based methods were used. Our data indicate that the dorsal surface of

the tongue serves as a potential reservoir for bacterial species involved in VAP. Furthermore, it is clear that the diversity of bacterial pathogens for VAP is far more complex than the current literature suggests.

- ³³ Paju S, Bernstein JM, Haase EM, Scannapieco FA. Molecular analysis of bacterial flora associated with chronically inflamed maxillary sinuses. *J Med Microbiol* 2003; 52: 591-7.

Abstract

*Chronic maxillary sinusitis is a chronic inflammatory condition in which the role of microbial infection remains undefined. Bacteria have been isolated from chronically inflamed sinuses; however, their role in the chronicity of inflammation is unknown. The objective of this study was to determine whether bacteria are present in clinical samples from chronic maxillary sinusitis and to assess the diversity of the flora present. Washes and/or tissue samples from endoscopic sinus surgery on 11 patients with chronic maxillary sinusitis were subjected to PCR amplification of bacterial 16S rDNA using three universal primer pairs, followed by cloning and sequencing. The samples were also assessed for the presence of bacteria and fungi by conventional culture methods. Viable bacteria and/or bacterial 16S rDNA were detected from maxillary sinus samples of five of the 11 patients examined (45 %). Three sinus samples were positive by both PCR and culture methods, one was positive only by PCR, and one only by culture. Thirteen bacterial species were identified: *Abiotrophia defectiva*, *Enterococcus avium*, *Eubacterium sp.*, *Granulicatella elegans*, *Neisseria sp.*, *Prevotella sp.*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, *Stenotrophomonas maltophilia*, *Streptococcus gordonii*, *Streptococcus mitis*/*Streptococcus oralis* and *Streptococcus sp.* Fungi were not detected. In one patient *Streptococcus mitis*/*Streptococcus oralis*, and in another patient *Pseudomonas aeruginosa*, were detected from both the sinus and the oral cavity using species-specific PCR primers. These results suggest that both aerobic and anaerobic bacteria can be detected in nearly half of chronic maxillary sinusitis cases.*

- ³⁴ Bittar F, Richet H, Dubus JC, Reynaud-Gaubert M, Stremmer N, Sarles J, et al. Molecular detection of multiple emerging pathogens in sputa from cystic fibrosis patients. *PLoS One* 2008; 3: e2908.

Abstract

BACKGROUND:

There is strong evidence that culture-based methods detect only a small proportion of bacteria present in the respiratory tracts of cystic fibrosis (CF) patients.

METHODOLOGY/PRINCIPAL FINDINGS:

*Standard microbiological culture and phenotypic identification of bacteria in sputa from CF patients have been compared to molecular methods by the use of 16S rDNA amplification, cloning and sequencing. Twenty-five sputa from CF patients were cultured that yield 33 isolates (13 species) known to be pathogens during CF. For molecular cloning, 760 clones were sequenced (7.2+/-3.9 species/sputum), and 53 different bacterial species were identified including 16 species of anaerobes (30%). Discrepancies between culture and molecular data were numerous and demonstrate that accurate identification remains challenging. New or emerging bacteria not or rarely reported in CF patients were detected including *Dolosigranulum pigrum*, *Dialister pneumosintes*, and *Inquilinus limosus*.*

CONCLUSIONS/SIGNIFICANCE:

Our results demonstrate the complex microbial community in sputa from CF patients, especially anaerobic bacteria that are probably an underestimated cause of CF lung pathology. Metagenomic analysis is urgently needed to better understand those complex communities in CF pulmonary infections.

- ³⁵ Han YW, Ikegami A, Bissada NF, Herbst M, Redline RW, Ashmead GG. Transmission of an uncultivated *Bergeyella* strain from the oral cavity to amniotic fluid in a case of preterm birth. *J Clin Microbiol* 2006; 44: 1475-83.

Abstract

Intrauterine infection is a recognized cause of preterm birth. The infectious organisms are believed to originate primarily from the vaginal tract and secondarily from other parts of the body. It is plausible that microbes in the oral cavity can be transmitted to the pregnant uterus. However, direct evidence supporting such a transmission is lacking. In this study, amniotic fluids of 34 pregnant women were examined by PCR using 16S and 23S rRNA universally conserved primers. Bacterial DNA was amplified from the only patient with clinical intrauterine infection and histologic necrotizing acute and chronic chorioamnionitis. One

strain, *Bergeyella* sp. clone AF14, was detected and was 99.7% identical to a previously reported uncultivated oral *Bergeyella* strain, clone AK152, at the 16S rRNA level. The same strain was detected in the subgingival plaque of the patient but not in her vaginal tract. The 16S-23S rRNA sequence of clone AF14 matched exactly with the sequences amplified from the patient's subgingival plaque. These observations suggest that the *Bergeyella* strain identified in the patient's intrauterine infection originated from the oral cavity. This is the first direct evidence of oral-utero microbial transmission. The patient's periodontal health during pregnancy was unclear. She did not have detectable periodontal disease during postpartum examination. *Bergeyella* spp. had not been previously associated with preterm birth and were detected in subgingival plaque of women without clinical levels of intrauterine infection. Uncultivated species may be overlooked opportunistic pathogens in preterm birth. This study sheds new light on the implication of oral bacteria in preterm birth.

³⁶ Nordquist, W.D. Oral Spirochetosis Associated with Dental Implants: Important Clues to Systemic Diseases, *Int'l Jour of Clin Implant Dent* Vol. 1 No. 1, Jan-Apr, 2009.

Abstract

The association between oral spirochetes and implant failure is documented. Microscopic evidence of spirochetal round "spore-like" forms are presented and discussed.

³⁷ Chukkappalli SS, Rivera MF, Velsko IM, Lee JY, Chen H, Zheng D, Bhattacharyya I, Gangula PR, Lucas AR, Kesavalu L. Invasion of oral and aortic tissues by oral spirochete *Treponema denticola* in ApoE(-/-) mice causally links periodontal disease and atherosclerosis. *Infect Immun*. 2014 May;82(5):1959-67. doi: 10.1128/IAI.01511-14. Epub 2014 Feb 24.

Abstract

Treponema denticola is a predominantly subgingival oral spirochete closely associated with periodontal disease and has been detected in atherosclerosis. This study was designed to evaluate causative links between periodontal disease induced by chronic oral *T. denticola* infection and atherosclerosis in hyperlipidemic ApoE(-/-) mice. ApoE(-/-) mice ($n = 24$) were orally infected with *T. denticola* ATCC 35404 and were euthanized after 12 and 24 weeks. *T. denticola* genomic DNA was detected in oral plaque samples, indicating colonization of the oral cavity. Infection elicited significantly ($P = 0.0172$) higher IgG antibody levels and enhanced intrabony defects than sham infection. *T. denticola*-infected mice had higher levels of horizontal alveolar bone resorption than sham-infected mice and an associated significant increase in aortic plaque area ($P \leq 0.05$). Increased atherosclerotic plaque correlated with reduced serum nitric oxide (NO) levels and increased serum-oxidized low-density lipoprotein (LDL) levels compared to those of sham-infected mice. *T. denticola* infection altered the expression of genes known to be involved in atherosclerotic development, including the leukocyte/endothelial cell adhesion gene (*Thbs4*), the connective tissue growth factor gene (*Ctgf*), and the selectin-E gene (*Sele*). Fluorescent in situ hybridization (FISH) revealed *T. denticola* clusters in both gingival and aortic tissue of infected mice. This is the first study examining the potential causative role of chronic *T. denticola* periodontal infection and vascular atherosclerosis in vivo in hyperlipidemic ApoE(-/-) mice. *T. denticola* is closely associated with periodontal disease and the rapid progression of atheroma in ApoE(-/-) mice. These studies confirm a causal link for active oral *T. denticola* infection with both atheroma and periodontal disease.

³⁸ Siqueira Jr JF. and Rocas IN. As-yet-uncultivated oral bacteria: breadth and association with oral and extra-oral diseases. *Journal of Oral Microbiology* 2013, 5: 21077.

Abstract

It has been shown that 40-60% of the bacteria found in different healthy and diseased oral sites still remain to be grown in vitro, phenotypically characterized, and formally named as species. The possibility exists that these as-yet-uncultivated bacteria play important ecological roles in oral bacterial communities and may participate in the pathogenesis of several oral infectious diseases. There is also a potential for these as-yet-uncultivated oral bacteria to take part in extra-oral infections. For a comprehensive characterization of physiological and pathogenic properties as well as antimicrobial susceptibility of individual bacterial species, strains need to be grown in pure culture. Advances in culturing techniques have allowed the cultivation of several oral bacterial taxa only previously known by a 16S rRNA gene sequence signature, and novel species have been proposed. There is a growing need for developing

improved methods to cultivate and characterize the as-yet-uncultivated portion of the oral microbiome so as to unravel its role in health and disease.

- ³⁹ Siqueira JF, Jr., Roças IN. Diversity of endodontic microbiota revisited. *J Dent Res* 2009; 88: 96981.

Abstract

Although fungi, archaea, and viruses contribute to the microbial diversity in endodontic infections, bacteria are the most common micro-organisms occurring in these infections. Datasets from culture and molecular studies, integrated here for the first time, showed that over 460 unique bacterial taxa belonging to 100 genera and 9 phyla have been identified in different types of endodontic infections. The phyla with the highest species richness were Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. Diversity varies significantly according to the type of infection. Overall, more taxa have been disclosed by molecular studies than by culture. Many cultivable and as-yet-uncultivated phylotypes have emerged as candidate pathogens based on detection in several studies and/or high prevalence. Now that a comprehensive inventory of the endodontic microbial taxa has been established, future research should focus on the association with different disease conditions, functional roles in the community, and susceptibility to antimicrobial treatment procedures.

- ⁴⁰ Sakamoto M, Roças IN, Siqueira JF, Jr, Benno Y. Molecular analysis of bacteria in asymptomatic and symptomatic endodontic infections. *Oral Microbiol Immunol* 2006; 21: 11222.

Abstract

*The purpose of the present study was to use terminal restriction fragment length polymorphism analysis and the 16S rRNA gene clone library to investigate the diversity of the microbiota associated with asymptomatic and symptomatic endodontic infections and to compare the bacterial community structure in these two clinical conditions. Samples were taken from asymptomatic endodontic infections associated with chronic periradicular lesions and from symptomatic infections clinically diagnosed as acute abscesses. 16S rRNA genes from DNA isolated from clinical samples were used to construct clone libraries or were subjected to terminal restriction fragment length polymorphism analysis. Sequence analysis of 186 clones revealed 42 taxa; 23 (55%) were uncultivated phylotypes, of which seven were unique to endodontic infections. Clone sequencing and terminal restriction fragment length polymorphism analysis revealed that the most commonly detected taxa were *Fusobacterium nucleatum* (including terminal restriction fragment types 1 and 2), *Peptostreptococcus micros/Peptostreptococcus sp. oral clone AJ062/BS044/FG014*, *Prevotella species*, *Dialister species*, *Mogibacterium species*, *Lachnospiraceae oral clone 55A-34*, *Filifactor alocis*, *Megasphaera sp. oral clone CS025/BS073*, and *Veillonella sp. oral clone BP1-85/Veillonella dispar/V. parvula*. *Bacteroides-like sp. oral clone X083/Bacteroidales oral clone MCE7_20* and *Dialister sp. oral clone BS016/MCE7_134* were detected only in asymptomatic teeth. On the other hand, *F. nucleatum* terminal restriction fragment type 2, *Prevotella intermedia*, *Dialister pneumosintes*, and some phylotypes were exclusively detected in symptomatic samples. Bacterial profiles of symptomatic endodontic infections generated by terminal restriction fragment length polymorphism analysis were clearly different from those of asymptomatic infections. Overall, the average number of terminal restriction fragments in symptomatic samples was significantly larger than in asymptomatic samples. Molecular analysis of the microbiota associated with symptomatic or asymptomatic endodontic infections indicates that the endodontic bacterial diversity is greater than previously described by culture methods and that the structure of the microbiota differ significantly between asymptomatic and symptomatic infections.*

- ⁴¹ Flynn TR, Paster BJ, Stokes LN, Susarla SM, Shanti RM. Molecular methods for diagnosis of odontogenic infections. *J Oral Maxillofac Surg* 2012; 70: 18549.

Abstract

PURPOSE:

Historically, the identification of microorganisms has been limited to species that could be cultured in the microbiology laboratory. The purpose of the present study was to apply molecular techniques to identify microorganisms in orofacial odontogenic infections (OIs).

MATERIALS AND METHODS:

Specimens were obtained from subjects with clinical evidence of OI. To identify the microorganisms involved, 16S rRNA sequencing methods were used on clinical specimens. The name and number of the

clones of each species identified and the combinations of species present were recorded for each subject. Descriptive statistics were computed for the study variables.

RESULTS:

Specimens of pus or wound fluid were obtained from 9 subjects. A mean of 7.4 ± 3.7 (standard deviation) species per case were identified. The predominant species detected in the present study that have previously been associated with OIs were *Fusobacterium* spp, *Parvimonas micra*, *Porphyromonas endodontalis*, and *Prevotella oris*. The predominant species detected in our study that have not been previously associated with OIs were *Dialister pneumosintes* and *Eubacterium brachy*. Unculturable phylotypes accounted for 24% of the species identified in our study. All species detected were obligate or facultative anaerobes. Streptococci were not detected.

CONCLUSIONS:

Molecular methods have enabled us to detect previously cultivated and not-yet-cultivated species in OIs; these methods could change our understanding of the pathogenic flora of orofacial OIs.

⁴² Sakamoto M, Rôças IN, Siqueira JF, Jr, Benno Y. Molecular analysis of bacteria in asymptomatic and symptomatic endodontic infections. *Oral Microbiol Immunol* 2006; 21: 11222.

Abstract

The purpose of the present study was to use terminal restriction fragment length polymorphism analysis and the 16S rRNA gene clone library to investigate the diversity of the microbiota associated with asymptomatic and symptomatic endodontic infections and to compare the bacterial community structure in these two clinical conditions. Samples were taken from asymptomatic endodontic infections associated with chronic periradicular lesions and from symptomatic infections clinically diagnosed as acute abscesses. 16S rRNA genes from DNA isolated from clinical samples were used to construct clone libraries or were subjected to terminal restriction fragment length polymorphism analysis. Sequence analysis of 186 clones revealed 42 taxa; 23 (55%) were uncultivated phylotypes, of which seven were unique to endodontic infections. Clone sequencing and terminal restriction fragment length polymorphism analysis revealed that the most commonly detected taxa were *Fusobacterium nucleatum* (including terminal restriction fragment types 1 and 2), *Peptostreptococcus micros/Peptostreptococcus* sp. oral clone AJ062/BS044/FG014, *Prevotella* species, *Dialister* species, *Mogibacterium* species, *Lachnospiraceae* oral clone 55A-34, *Filifactor alocis*, *Megasphaera* sp. oral clone CS025/BS073, and *Veillonella* sp. oral clone BP1-85/*Veillonella dispar/V. parvula*. *Bacteroides*-like sp. oral clone X083/*Bacteroidales* oral clone MCE7_20 and *Dialister* sp. oral clone BS016/MCE7_134 were detected only in asymptomatic teeth. On the other hand, *F. nucleatum* terminal restriction fragment type 2, *Prevotella intermedia*, *Dialister pneumosintes*, and some phylotypes were exclusively detected in symptomatic samples. Bacterial profiles of symptomatic endodontic infections generated by terminal restriction fragment length polymorphism analysis were clearly different from those of asymptomatic infections. Overall, the average number of terminal restriction fragments in symptomatic samples was significantly larger than in asymptomatic samples. Molecular analysis of the microbiota associated with symptomatic or asymptomatic endodontic infections indicates that the endodontic bacterial diversity is greater than previously described by culture methods and that the structure of the microbiota differ significantly between asymptomatic and symptomatic infections.

⁴³ Rôças IN, Siqueira JF Jr, Santos KR, Coelho AM.

"Red complex" (*Bacteroides forsythus*, *Porphyromonas gingivalis*, and *Treponema denticola*) in endodontic infections: a molecular approach. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2001 Apr;91(4):468-71.

Abstract

OBJECTIVE:

The "red complex," composed of *Bacteroides forsythus*, *Porphyromonas gingivalis*, and *Treponema denticola*, is implicated in severe forms of periodontal diseases. The purpose of this study was to assess the occurrence of the red complex in root canal infections through the use of a sensitive technique-the 16S rDNA-directed polymerase chain reaction (PCR).

STUDY DESIGN:

Samples were obtained from 50 necrotic pulps with periradicular pathosis. Ten cases were diagnosed as acute periradicular abscesses. DNA was extracted from the samples and analyzed with a PCR-based identification assay.

RESULTS:

At least 1 member of the red complex was found in 33 of 50 cases. *T denticola*, *P gingivalis*, and *B forsythus* were detected in 44%, 30%, and 26% of the cases, respectively. The red complex was found in 4 of 50 cases. No particular signs or symptoms were associated with the presence of these bacterial species.

CONCLUSIONS:

Despite what is indicated in reports with respect to marginal periodontitis, red complex bacteria-either singularly or collectively-was not associated with any particular pattern of clinical symptoms. However, because the bacterial species from the red complex are recognized oral pathogens, their occurrence in root canal infections suggests that they may play a role in the pathogenesis of periradicular diseases.

⁴⁴ Saber MH1, Schwarzberg K, Alonaizan FA, Kelley ST, Sedghizadeh PP, Furlan M, Levy TA, Simon JH, Slots J. Bacterial flora of dental periradicular lesions analyzed by the 454-pyrosequencing technology. *J Endod.* 2012 Nov;38(11):1484-8

Abstract

INTRODUCTION:

Symptomatic teeth with periradicular lesions of infectious origin remain a significant challenge in dentistry, and the reason for the acute perturbation is incompletely understood. The present study used pyrosequencing of bacterial 16S ribosomal RNA (rRNA) genes to characterize the microbiota of periradicular lesions.

METHODS:

Thirteen periradicular lesions from 11 symptomatic and 2 asymptomatic teeth were sampled during apical surgery. Samples were subjected to DNA extraction and 16S rRNA polymerase chain reaction (PCR) amplification. PCR amplicons were then sequenced by using the Roche 454 GS FLX platform. Data were analyzed with the Quantitative Insights into Microbial Ecology (QIIME) software package.

RESULTS:

Seven of the 13 periradicular lesions (53.8%) yielded PCR amplicons, which generated 35,731 high-quality DNA sequences belonging to 10 bacterial phyla and 73 bacterial genera. All 7 lesions were associated with symptoms. The phyla with most bacterial taxa were Proteobacteria (proportion of total bacterial taxa, 33.3%), Firmicutes (30.9%), Actinobacteria (12.2%), and Bacteroidetes (11.4%). The most abundant genera were *Fusobacterium* (average of total sequences, 21.0%), *Streptococcus* (8.0%), *Prevotella* (7.5%), *Corynebacterium* (7.2%), *Porphyromonas* (6.0%), and *Actinomyces* (5.8%).

CONCLUSIONS:

This study demonstrated that the microbiota of symptomatic periapical lesions is predominated by anaerobic bacteria but also contains substantial levels of streptococci, actinomyces, and bacteria not previously identified in the oral cavity. The etiopathogenic role and therapeutic implication of periradicular bacteria need to be determined.

⁴⁵ Gargiulo S, Gamba P, Poli G, Leonarduzzi G. Metalloproteinases and metalloproteinase inhibitors in age-related diseases. *Curr Pharm Des.* 2014;20(18):2993-3018.

Abstract

Degradation of the extracellular matrix is an important feature of embryonic development, morphogenesis, angiogenesis, tissue repair and remodeling. It is precisely regulated under physiological conditions, but when dysregulated it becomes a cause of many diseases, including atherosclerosis, osteoarthritis, diabetic vascular complications, and neurodegeneration. Various types of proteinases are implicated in extracellular matrix degradation, but the major enzymes are considered to be metalloproteinases such as matrix metalloproteinases (MMPs) and disintegrin and metalloproteinase domain (ADAMs) that include ADAMs with a thrombospondin domain (ADAMTS). This review discusses involvement of the major metalloproteinases in some age-related chronic diseases, and examines what is currently known about the beneficial effects of their inhibitors, used as new therapeutic strategies for treating or preventing the development and progression of these diseases.

⁴⁶ Ellen RP1, Galimanas VB. Spirochetes at the forefront of periodontal infections. *Periodontol* 2000. 2005;38:13-32.

⁴⁷ Brynolf I. A histological and roentgenological study of the periapical region of upper incisors. *Odont Revy*. 1967;(11):1-97.

Abstract

Purpose/Objectives: To compare the histological and roentgenological appearance of periapical changes in an attempt to find out whether the extent of histological changes are reflected in conventional roentgenograms.

Materials & Methods: 292 maxillary incisors obtained from cadavers were used. Immediately after the roentgenograms were taken, the tips of the roots were removed with a trepan. The specimens were fixed and prepared histologically. Specimens were then divided into 3 groups depending on the inflammatory cells in apical tissue, type of periapical marrow, and the shape of apical soft tissue. Group N (normal group), without inflammatory cells in apical soft tissue. Group M (marginal group), with very few scattered inflammatory cells and increased width of apical soft tissue. Group P, with mild to severe chronic inflammation cells in the apical soft tissue. Details in the roentgenograms were defined, graded and recorded in the same way as in the histological study.

Results: It was found that the changes in the roentgenological groups are very similar with those in the histological groups.

Author's Conclusion: Careful examination of high quality roentgenograms of the periapical areas of the upper incisors allows differentiation between histologically normal and pathological cases, and classification of different types and stages of histologic periapical lesions.

⁴⁸ Green T, Walton R, Merrell P. Radiographic and histologic periapical findings of root canal treated teeth in cadaver. *Oral surg Oral Med Oral Pathol Radiol Endol* 1997;83:707-11.

Abstract

The success of root canal treatment can be subjectively evaluated both clinically and radiographically. Normally, the recall radiograph is the main factor in evaluating success or failure.

OBJECTIVES:

This study evaluated periapical areas of root canal treated teeth by correlating radiographic and histologic findings.

STUDY DESIGN:

Jaws were resected from cadavers and radiographed. Those teeth that had received root canal treatment were evaluated for success or failure based on radiographic criteria. Teeth and surrounding bone were then removed en bloc and prepared for light microscopy. Untreated teeth without periapical pathosis were examined as controls.

RESULTS:

Root canal treated teeth classified as failures were found to consistently have inflammatory resorptive lesions at the periapices. In contrast, those treated teeth classified as radiographically successful showed varying reactions ranging from normal uninflamed to mildly inflamed.

CONCLUSIONS:

Those classified as failure showed consistent inflammation. However, the majority of our examined treated teeth were radiographically normal and exhibited no periapical inflammation.

⁴⁹ Inagaki S, Kimizuka R, Kokubu E, Saito A, Ishihara K. *Treponema denticola* invasion into human gingival epithelial cells. *Microb Pathog*. 2016 May;94:104-11.

Abstract

*Host cell invasion is important for periodontal pathogens in evading host defenses and spreading into deeper areas of the periodontal tissue. *Treponema denticola* has been implicated in a number of potentially pathogenic processes, including periodontal tissue penetration. Here we tested the ability of *T. denticola* strains to invade human gingival epithelial cells (HGEC). After 2 h infection, intracellular location of *T. denticola* cells was confirmed by confocal laser scanning microscopy (CLSM). Results from an antibiotic protection assay following [(3)H]uridine labeling indicated that invasion efficiency reached a maximum at 2 h after infection. Internalized *T. denticola* cells were still observed in HGEC at 24 h by CLSM.*

*A dentilisin deficient mutant exhibited significantly decreased invasion ($p < 0.05$) compared with the wild-type strain. In inhibition assays, phenylmethylsulfonyl fluoride and metabolic inhibitors such as methyl- β -cyclodextrin and staurosporine significantly reduced *T. denticola* invasion. Under CLSM, *T. denticola* colocalized with GM-1 ganglioside-containing membrane microdomains in a cholesterol-dependent manner. These results indicated that *T. denticola* has the ability to invade into and survive within HGECs. Dentilisin activity of *T. denticola* and lipid rafts on HGEC appear to play important roles in this process.*

⁵⁰ Personal communication with leading scientists working in a leading DNA analysis laboratory—details cannot be disclosed.

⁵¹ Domb WC. Ozone therapy in dentistry. A brief review for physicians. Interv Neuroradiol. 2014 Oct 31;20(5):632-6.

The 21st century dental practice is quite dynamic. New treatment protocols and new materials are being developed at a rapid pace. Ozone dental therapy falls into the category of new treatment protocols in dentistry, yet ozone is not new at all. Ozone therapy is already a major treatment modality in Europe, South America and a number of other countries. What is provided here will not be an exhaustive scientific treatise so much as a brief general introduction into what dentists are now doing with ozone therapies and the numerous oral/systemic links that make this subject so important for physicians so that, ultimately, they may serve their patients more effectively and productively.