

Roles of Oral Bacteria in Cardiovascular Diseases — From Molecular Mechanisms to Clinical Cases:

Implication of Periodontal Diseases in Development of Systemic Diseases

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Abstract. Periodontal diseases, some of the most common infectious diseases seen in humans, are characterized by gingival inflammation, as well as loss of connective tissue and bone from around the roots of the teeth, which leads to eventual tooth exfoliation. In the past decade, the association of periodontal diseases with the development of systemic diseases has received increasing attention. Although a number of studies have presented evidence of close relationships between periodontal and systemic diseases, the majority of findings are limited to epidemiological studies, while the etiological details remain unclear. Nevertheless, a variety of recent hypothesis driven investigations have compiled various results showing that periodontal infection and subsequent direct oral-hematogenous spread of bacteria are implicated in the development of various systemic diseases. Herein, we present current understanding in regard to the relationship between periodontal and systemic diseases, including cardiovascular diseases, preterm delivery of low birth weight, diabetes mellitus, respiratory diseases, and osteoporosis.

Keywords: periodontal disease, diabetes mellitus, cardiovascular disease, preterm delivery, *Porphyromonas gingivalis*, oral bacteria

1. Introduction

Epidemiological and interventional studies of humans have revealed close associations between periodontal diseases and systemic diseases, such as cardiovascular disease (CVD) (1 – 8), preterm delivery of low birth weight (PTLBW) (9 – 14), diabetes mellitus (15 – 18), respiratory infection (19), and osteoporosis (20, 21). From those results, it is not an exaggeration to state that periodontal infection represents a significant risk factor for various systemic diseases. *Porphyromonas gingivalis* has been reported to be involved in the development of systemic diseases due to systemic inflammation with increased circulating cytokines and mediators, direct infection, and cross-reactivity/molecular mimicry between bacterial antigens and self-antigens (22). Furthermore, periodontal pathogens including *P. gingivalis* have been

detected in heart valve lesions and atheromatous plaque (5, 6), amniotic fluid of pregnant women with threatened premature labor (11), and placentas from cases of preterm delivery (12, 13). Although a definitive role for *P. gingivalis* in systemic diseases has yet to be established, the involvement of the bacterium in these disorders has been strongly suggested.

This review provides an overview of the relationship between chronic periodontitis and systemic diseases, including CVD, PTLBW, diabetes mellitus, respiratory diseases, and osteoporosis.

2. Periodontitis

Periodontal (gum) diseases, including gingivitis and chronic periodontitis, are serious infections that, left untreated, can lead to tooth loss. Periodontal disease has been proposed to affect at least one tooth in 80% of adults worldwide (23), with the main cause shown to be bacterial plaque (also called biofilm), which is a sticky, colorless film that constantly forms on teeth (Fig. 1). Gingivitis

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is manifested by red, swollen gums and bleeding that may occur with tooth brushing and can advance to periodontitis when untreated. Over time, plaque biofilm can spread and grow below the gum line (subgingival area), and toxins produced by the bacteria in plaque biofilm irritate the gums. Those toxins stimulate a chronic inflammatory response in which the body, in essence, turns on itself, and the tissues and bone that support the teeth are broken down and destroyed. In this process, gums separate from the teeth, forming periodontal pockets (spaces between teeth and gums) that become infected. As the disease progresses, the pockets deepen and more gum tissue and bone are destroyed. Often, this destructive process has very mild symptoms, though teeth can eventually become loose and may have to be removed. In developed countries, chronic periodontitis has been estimated to affect up to 22% of the adult population, with moderate and severe forms affecting 13% (23). Although the etiology of chronic periodontitis is multifactorial, evidence exists indicating that specific Gram-negative bacteria in subgingival plaque biofilm play an important role in progression of the disease (23). The oral cavity supports more than 700 different bacterial species and the periodontal pocket area harbors more than 400 species. Among the various subgingival plaque bacterial species, substantial evidence indicates roles for 3 species, *P. gingivalis*, *Treponema denticola*, and *Tannerella forsythia*, in initiation and progression of the disease, with *P. gingivalis* implicated as a major etiological agent (24).

3. Oral-hematogenous spread of bacteria

In the oral cavity, the innate host defense system is thought to act toward limiting the spread of oral bacteria by maintaining intact gingival epithelium, an innate physical barrier. The epithelium is also important in orchestrating host inflammatory responses (25). However, as the disease progresses, the epithelium becomes ulcerated to expose the underlying connective tissues and blood capillaries to plaque biofilm. The exposed ulcerative area, which has an area ranging from 8 – 20 cm² in an oral cavity with medium periodontitis (26, 27), facilitates direct entry of biofilm organisms to the circulation during eating and tooth brushing. This oral-hematogenous spread of bacteria is thought to be a main cause of periodontitis-related systemic diseases (Fig. 2). Furthermore, peripheral polymorphonuclear leukocytes (PMNLs) from patients with periodontitis have been reported to be hyper-reactive as compared to controls with respect to the release of reactive oxygen species (28) and PMNL-specific proteases (28). In addition, the levels of inflammatory markers are also elevated (29) and antioxidant defenses

appear to be compromised (30). It should be noted that traditional anti-infective periodontal therapies, such as scaling and root planing, with or without adjunctive antibiotics, not only improve the clinical symptoms of periodontitis, but also improve local antioxidant status (30) and systemic vascular endothelial dysfunction (31) and reduce PMNL hyper-reactivity (28).

4. CVD

CVD refers to a group of disorders of the heart and vasculature and includes high blood pressure, coronary heart disease (CHD), congestive heart failure, stroke, and myocardial infarction (32). These diseases account for approximately 17 million deaths per annum and 40% of all deaths worldwide, with atherosclerosis the underlying etiology in the vast majority of cases (32). In addition to traditional exacerbation factors, such as smoking, hypercholesterolemia, hypertension, and diabetes mellitus (32, 33), periodontitis has been shown to be linked to the development of atherosclerosis (23). Recently, an Editors' consensus regarding "Periodontitis and Atherosclerotic CVD" presented in the *American Journal of Cardiology* and *Journal of Periodontology* noted a significant relationship between periodontitis and atherosclerotic CVD (34, 35).

The relationship between CHD and periodontitis was first reported at the end of the 1980s (36). Periodontal health is a significant predictor of coronary atheromatosis along with the following parameters: age, sex, socioeconomic status, smoking, hypertension, number of previous myocardial infarctions, diabetes mellitus, body mass index, and serum lipid levels (1). Since a significant epidemiological relationship was known at that time, etiological investigations were not performed to determine whether oral infection is a risk factor for CVD or if oral bacteria directly affect cardiovascular condition. However, in the recent decade, the epidemiological causal association between periodontal disease and CHD has been reconsidered (2), with meta-analysis revealing that periodontal disease is likely associated with the risk of future CHD, which is increased in individuals older than 65 years (3).

Bacterial DNAs of periodontal pathogens, such as *P. gingivalis*, *T. forsythia*, *T. denticola*, *Aggregatibacter actinomycetemcomitans*, and *Campylobacter rectus*, have been detected in stenotic coronary artery plaque samples (4) and aneurysmal wall and aneurysmal thrombus tissues (5). In that latter study, patients with Buerger disease had significantly higher serum IgG titers against *T. denticola*, *P. gingivalis*, and *A. actinomycetemcomitans*, while DNAs of those bacteria have been detected in occluded artery tissues (6). In addition, the occurrence of

P. gingivalis in stenotic coronary artery plaque specimens from patients with severe periodontitis was 5-fold greater than that in those from patients with medium periodontitis (4). However, those results are not conclusive to directly imply that periodontal pathogens in lesions are directly implicated in the formation of atherogenic plaque. Thus, animal experiments were performed, which clearly indicated that oral infection with *P. gingivalis* exacerbated atheromatosis (37–39). ApoE-deficient mice with a high-fat diet had an accelerated atherogenic plaque progression of atherosclerosis when infected with *P. gingivalis* via oral challenge (37, 38). In addition, *P. gingivalis* was shown to localize in various tissues by polymerase chain reaction (PCR) analysis, while marked levels of *P. gingivalis*-specific IgG in serum were also reported (39). Those results demonstrated that *P. gingivalis* organisms can penetrate gingival tissues and enter the bloodstream and then play a direct or indirect role in the progression of atherosclerosis.

An important feature in the development of early atherosclerotic lesions is cholesterol uptake by macrophages to form foam cells as well as lipid accumulation in sub-endothelial spaces (7). Foam cells also stimulate the

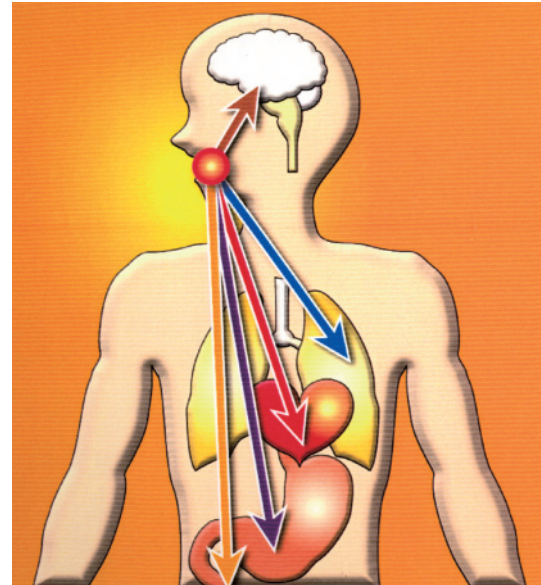


Fig. 2. Cause of periodontitis-related systemic diseases. The gingival epithelium functions as an innate physical barrier to protect periodontal tissues from bacterial invaders. However, with disease progression, local inflammation ulcerates the epithelium to expose the underlying connective tissues and blood capillaries to plaque biofilm. The exposed ulcerative area (8–20 cm² in affected oral cavities) facilitates direct entry of biofilm pathogens into the circulation during eating and tooth brushing. Eventually, periodontal pathogens are able to migrate throughout the entire body. This oral-hematogenous spread of bacteria is a primary cause of periodontitis-derived systemic diseases.

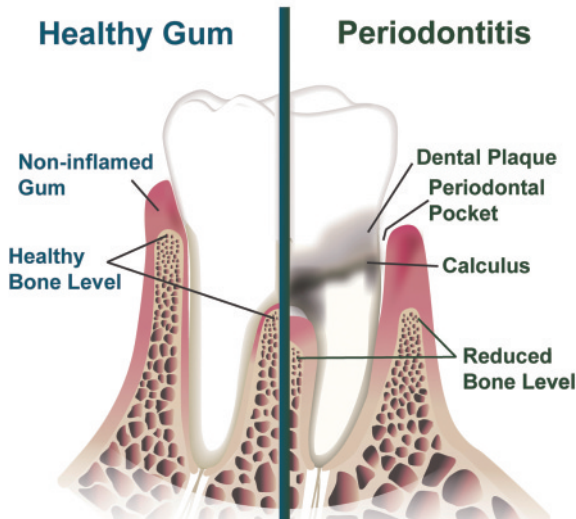


Fig. 1. Cause of periodontitis. The main cause of periodontitis is bacterial plaque (also called biofilm), a sticky, colorless film that constantly forms on teeth. Over time, plaque biofilm can spread and grow below the gum line, and toxins produced by the bacteria in the plaque biofilm irritate the gums. The toxins stimulate host inflammatory responses that induce destruction of the epithelial and connective tissues, as well as bone supporting the teeth. Thereafter, the gums separate from the teeth and form periodontal pockets (spaces between teeth and gums), which harbor various periodontal pathogens. As the disease progresses, the pockets deepen and additional gum tissue and bone are destroyed (1). The illustration was kindly drawn by Shu Yoshimori (Kobe Design University, Kobe, Japan).

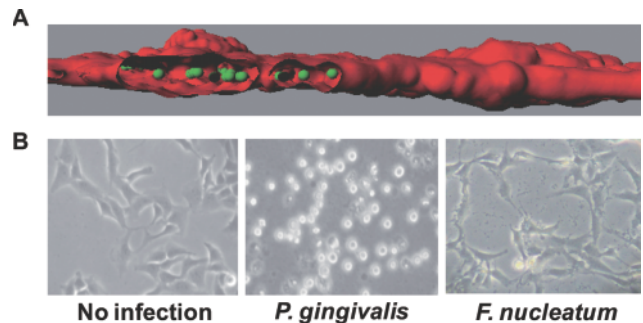


Fig. 3. *P. gingivalis* internalizes and impairs trophoblasts. A: Fluorescent confocal microscopic image of trophoblasts (red) infected with *P. gingivalis* (green) at a multiplicity of infection (moi) of 100 for 2 h. This 3-dimensional image was created with Imaris software. B: *P. gingivalis* infection induces morphological changes and inhibits cell proliferation in trophoblasts. The light microscopic images show the morphology of trophoblasts infected with *P. gingivalis* at a moi of 200 as well as with *Fusobacterium nucleatum* at an moi of 200 for 24 h. *F. nucleatum* is a Gram-negative oral bacterial species associated with periodontal disease progression, but is not considered as a bona-fide periodontal pathogen (24). Control cells were not infected. (Reproduced with permission from Ref. 14.)

production of pro-inflammatory cytokines, leading to further progression of atherosclerosis. Low-density lipoprotein (LDL) reportedly binds to specific proteins of *P. gingivalis* and the aggregate was found to induce murine macrophages to form foam cells (8). That finding also indicates a possible mechanism by which *P. gingivalis* causes atherosclerosis in the bloodstream. Furthermore, our recent study demonstrated novel cholesterol-independent mechanisms that are involved in the formation of vascular diseases associated with *P. gingivalis* (40, 41). The details of those mechanisms are discussed in the following reviews included in this volume (42, 43).

5. Preterm delivery of low birth weight

Preterm deliveries occur at a gestational age less than 37 weeks, except in cases of low-gestational age cutoff or spontaneous abortion (44). Bacterial infection is one of the major causes of PTLBW and reported to be associated with the presence of *Mycoplasma species*, especially *Ureaplasma urealyticum*, and many other organisms within the amnion, chorion, amniotic cavity, and the fetus (44). Such local bacterial infections result in bacterial vaginosis and chorioamnionitis, which can lead to spontaneous preterm birth in early gestation (23, 44). Recently, periodontitis has been investigated as a possible cause of PTLBW, and various epidemiological studies have shown a link between periodontal health and the condition (9), with odds ratios ranging from 1.1 to 20 shown by meta-analyses, though not all studies of this complex phenomenon have found an association (10).

Periodontal pathogens including *P. gingivalis* were detected using a PCR assay in the amniotic fluid of pregnant women with a diagnosis of threatened premature labor (11) as well as in placentas of women with preeclampsia (12). In an immunocytochemistry examination, localization of *P. gingivalis* was also shown in placental tissues, including syncytiotrophoblasts, chorionic trophoblasts, decidual cells, and amniotic epithelial cells, as well as vascular cells, which were obtained from women with chorioamnionitis at fewer than 37 weeks of gestation (13). *P. gingivalis* organisms were continuously infected via capillary vessels in the dorsolumbar region of a rabbit, after which *P. gingivalis* was found to achieve transplacental passage (45). *P. gingivalis* was also reported to invade both maternal and fetal tissues of rats, which resulted in chorioamnionitis and placentitis (46). Furthermore, *P. gingivalis* was shown to invade placental trophoblasts and induce apoptosis by cell cycle arrest (14), as shown in Fig. 3. Although additional investigations are necessary to establish the etiological correlation of PTLBW with periodontitis, oral-hematogenous spread

of *P. gingivalis* seems to be one of the causes of the condition. Therefore, we propose therapeutic elimination/suppression of *P. gingivalis* before pregnancy as an important factor for normal delivery.

6. Diabetes mellitus

Diabetes mellitus is a condition in which the body either does not produce enough, or does not properly respond to, insulin, a hormone produced in the pancreas. Insulin enables cells to absorb glucose in order to turn it into energy. In diabetes mellitus, the body either fails to properly respond to its own insulin, does not make enough insulin, or both. This causes glucose to accumulate in the blood, often leading to various complications such as acute/chronic infection, vascular diseases, neuropathy, nephropathy, and retinopathy (47). The number of adults throughout the world with diabetes mellitus is predicted to nearly double over the next 25 years, from approximately 171 million in 2000 to 366 million by 2030 (47). Periodontal disease is a potential complication of diabetes mellitus (23) and the chronic nature of that infection is considered to contribute to a worsening diabetic status (48), while successful periodontal treatment has been suggested to improve diabetes metabolic control (49). In contrast, a recent report based on meta-analysis of data obtained in 10 intervention trials (total of 456 patients) revealed that the decrease in glycated hemoglobin A1c (HbA1c) following periodontal therapy was not statistically significant (50).

Type 1 diabetes mellitus (previously known as insulin-dependent or childhood-onset diabetes) is characterized by a lack of insulin production. Type 2 diabetes mellitus (formerly called non-insulin-dependent or adult-onset diabetes) is caused by the body's ineffective use of insulin. It often results from excess body weight and physical inactivity (47). Type 1 diabetes mellitus has been suggested to increase the susceptibility to periodontitis, particularly in cases with poor metabolic control and/or diabetic complications (16). In another study, regardless of a significant improvement in periodontal infection after periodontal therapy, a majority of the patients reportedly showed negligible improvement in HbA1c findings (16). In type II diabetes mellitus, serum inflammatory cytokines, especially tumor necrosis factor (TNF)- α , have effects on insulin sensitivity, while those cytokines induced by periodontitis are considered to be related to metabolic abnormalities associated with diabetes mellitus (49). *P. gingivalis* and its components, including fimbriae and lipopolysaccharide, reportedly activate various host cells, resulting in the release of cytokines such as interleukin (IL)-1, IL-6, IL-8, and TNF- α (51). Successful periodontal treatment has been

suggested to improve diabetes metabolic control, possibly through reduced TNF- α and improved insulin resistance (49).

It is important to monitor HbA1c levels to assess diabetic management, as poor glycemic control over time has been linked to the development and progression of microvascular diabetic complications (17). Interestingly, *P. gingivalis* was detected more frequently in subjects with increased HbA1c levels after periodontal treatment than in those with decreased levels. Furthermore, *P. gingivalis* organisms with type II fimbriae were detected only in subjects with elevated HbA1c, while improvements in HbA1c levels were observed only in subjects without type II clones. These results suggest that glycemic level in diabetes mellitus is affected by the persistence of *P. gingivalis* in periodontal pockets, especially clones with type II fimbriae (18). Serum advanced glycation end products (AGEs) were also shown to be significantly associated with deterioration caused by periodontitis (15) and may be a useful biomarker to assess periodontitis associated with diabetes mellitus.

7. Respiratory infection

Respiratory infections, such as pneumonia and certain chronic obstructive pulmonary diseases (COPDs), involve aspiration of bacteria from the oropharynx into the lower respiratory tract, due to a swallowing disorder (52). Many clinical studies have reported a linkage between periodontitis and respiratory diseases (19, 53), which suggests that aspiration of oral bacteria infects respiratory ducts. In addition, *P. gingivalis* has been detected in sputum samples from elderly subjects with aspiration pneumonia (53). Also, oral infection by this bacterium apparently caused local inflammation leading to severe bronchopneumonia and lung abscess in mice (54). Thus, oral pathogens including *P. gingivalis* are likely one of the causes of respiratory infectious diseases.

8. Osteoporosis

Osteoporosis is a disorder of the skeletal system characterized by weakened bone strength, which results in an increased risk of fracture (55). Since both osteoporosis and periodontitis are highly prevalent and markedly associated with aging (20), various studies have been performed to investigate the linkage between these diseases over the past decades (21). For example, bone mineral density in relationship to periodontal condition was examined in older or postmenopausal women in a number of studies (20, 21). In animal experiments, rats and mice were subjected to ovariectomy procedures and studied,

as osteopenic animal models are known to mimic the development of estrogen deficiency-induced osteopenia in humans (56). However, despite those various studies, no clear linkage between these diseases has been shown.

9. Conclusion

There is compelling evidence to indicate a close linkage between periodontitis and several systemic diseases, among which atherosclerosis and type 2 diabetes mellitus are accepted to be most likely related to periodontitis. These periodontitis-linked systemic diseases are likely caused by oral-hematogenous spread of oral bacteria, with *P. gingivalis* especially noted in several studies to have an etiological role. However, multiple mechanisms are considered to be involved in *P. gingivalis*-involved systemic diseases, with active investigations currently in progress. Nevertheless, there is clear evidence of an epidemiological association between periodontitis and several systemic diseases, as discussed above; hence, the control of oral disease is essential for prevention and management of these systemic conditions. In terms of medical economics, understanding of the relationship between periodontitis and systemic diseases has potential to change health policy, with ensuing economic benefits.

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