Literature Review

CHRONIC INFLAMMATORY PERIODONTAL DISEASE

A RISK FACTOR FOR CARDIOVASCULAR DISEASE AND ISCHEMIC STROKE?

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Abstract
This literature review discusses the findings of more recent studies investigating the relationship between chronic inflammatory periodontal disease and risk for cardiovascular disease and stroke. The intensity of inflammation in moderate and severe chronic periodontitis is clearly sufficient to induce a systemic response. The systemic response is commonly expressed by elevated serum levels of inflammatory mediators and acute-phase reactants like C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF-α), serum amyloid A, fibrinogen, and haptoglobin. The presence of chronic oral inflammation may enhance atherosclerotic pathogenesis through one or more mechanisms, such as stimulation of humoral and cell-mediated inflammatory pathways, bacteremia leading to direct interaction of periodontal pathogenic microbes with the arterial wall, and increases in circulating mediators of inflammation.

There are many clinical studies and investigations using animal models that, when collectively considered, indicate a significant association of periodontitis with cardiovascular and cerebrovascular diseases. Although a direct causal relationship remains to be demonstrated, it appears that at the very least, periodontitis represents a systemic inflammatory burden that facilitates atheroma formation, which may lead to a cardiovascular or cerebrovascular event.

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Key words: Periodontitis, inflammation, inflammatory mediators, bacteria, cardiovascular disease, ischemic stroke

Hujoel and colleagues[1] have calculated that among individuals with chronic periodontitis, the surface area of the dentogingival epithelium exposed to potential bacterial invasion and/or infiltration of antigenic microbial components ranges between 8 cm^2 and 20 cm^2. Thus, it is not surprising that a breach of this epithelial barrier is a common occurrence in chronic and aggressive periodontitis, and is likely to result in systemic dissemination of microbes, antigens, and mediators of inflammation.

Locally, bacteria and their byproducts of metabolism stimulate a cellular immune response represented by a dense infiltration of neutrophils, macrophages, and various lymphoid cells. These cells and the host connective tissue cells associated with the inflammatory lesion are stimulated to synthesize and release proinflammatory cytokines and prostanoids — interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF-α), prostaglandin E_2 (PGE_2), and various matrix metalloproteinases (MMPs) — which play a role in the destruction of alveolar bone and connective tissues that furnish support to the teeth.[2] In addition to being a major cause of adult tooth loss, recent studies suggest that chronic and aggressive periodontitis may constitute an independent risk factor for

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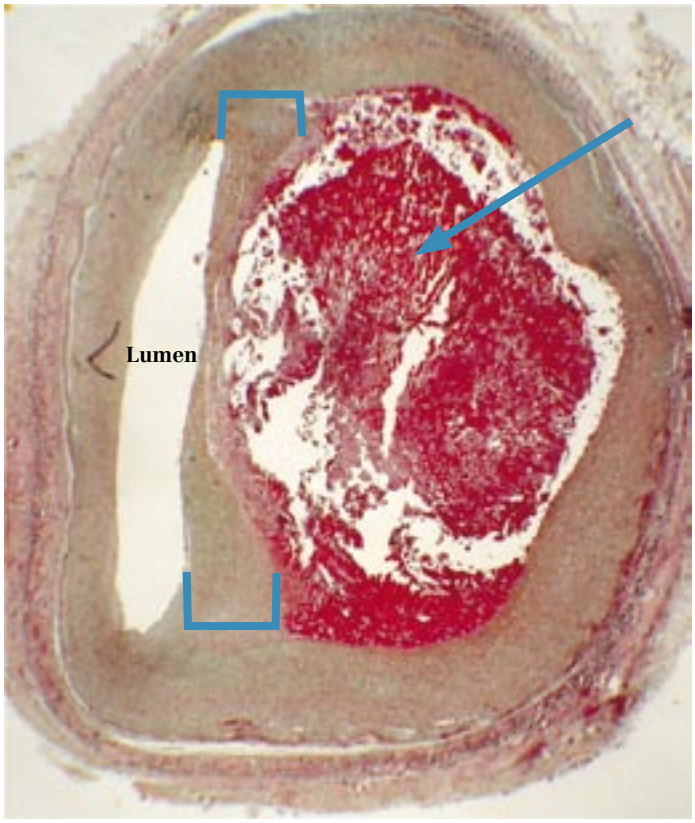

Figure 1

Histologic cross-section of a coronary artery exhibiting extensive atheroma formation consisting of fibrous cap (bracket) and infiltration of cholesterol (arrows).

Specimen provided by Dr. Joseph C. Whitt, Department of Oral and Maxillofacial Pathology, University of Missouri-Kansas City School of Dentistry, Kansas City, Mo. Movat's stain; original magnification x25.

cardiovascular disease and ischemic stroke.[3-14] However, other investigators have suggested that periodontitis may not be an independent risk factor but does represent a comorbid condition (i.e., a disease that coexists with other diseases because of a common causal factor). In some studies, smoking is shown as the common causal factor in periodontitis, cardiovascular disease, and ischemic stroke[15,16] In spite of the apparent differences in theory, periodontitis may add to the cumulative systemic insult derived from repeated exposures to other chronic inflammatory diseases during an individual's lifetime.[17-22]

Inflammatory markers

A variety of inflammation markers have been correlated to increasing severity of periodontitis, atherosclerosis, and ischemic stroke. For example, inflammation is characterized by the production of cell-derived mediators of inflammation, such as IL-6, TNF-α, and PGE_2. In turn, their systemic distribution via the vascular circulatory system induces the production of liver-derived markers of a systemic inflammatory reaction, such as c-reactive protein (CRP), serum amyloid A, fibrinogen, and haptoglobin. CRP is a particularly sensitive systemic marker of systemic inflammation. A serum CRP concentration of >10 mg/L is generally indicative of significant inflammatory disease. Compared with healthy controls, individuals with severe periodontitis are consistent in their expression of elevated serum CRP levels.[23-27] Other markers of inflammation elevated in cases of periodontitis, either in serum or gingival crevicular fluid, include haptoglobin,[23] fibrinogen,[27] serum amyloid A, IL-1, IL-6, IL-8, PGE_2, TNF-α, and various MMPs.[2]

Noack and colleagues[24] reported that the degree of increases in CRP levels in patients with periodontitis, when adjusted for confounding modifying factors, is dependent on the severity of the disease. The authors also demonstrated a strong relationship between elevated CRP levels and the presence of several periodontal pathogenic microbes, i.e., *Porphyromonas gingivalis (P. gingivalis), Prevotella intermedia (P. intermedia), Campylobacter rectus (C. rectus),* and *Tannerella forsythia (T. forsythia)*, thereby establishing an association between periodontal infections and elevated CRP levels. Serum levels of CRP, IL-6, fibrinogen, and IL-8 are also elevated in patients with unstable angina, myocardial infarction,[22] and ischemic stroke,[28] with higher levels being correlated to increasingly poor prognoses.[22]

Periodontal pathogenic microbes and vascular disease

Animal studies —

Following reports in medical literature that relate *Chlamydia pneumoniae* and cytomegalovirus infections to the etiology of atherosclerosis, Hzaraszthy and colleagues[29] reported that 40 of 50 (80 percent) endarterectomy specimens taken from patients with carotid stenosis were positive for periodontal pathogens, such as *T. forsythia, P. gingivalis, P. intermedia, or Actinobacillus actinomycetemcomitans (A. actinomycetemcomitans)*. In addition, almost 60 percent of the specimens were positive for two or more of the target microbes. Thus, their hypothesis that oral microbes associated with severe chronic periodontitis may gain access to the systemic circulatory system and, thereby, play a role in development of atherosclerosis was supported.

Additional support for this hypothesis soon followed in a series of animal studies. Using a mouse experimental model, Kesavalu and colleagues[30] were able to induce pro-inflammatory cytokine expression (IL-1β, IL-6, and TNF-α) following subcutaneous injection of *P. gingivalis* and *A. actinomycetemcomitans*. Li and colleagues[31] demonstrated that repeated systemic inoculation of *P. gingivalis* resulted in significant macrophage-rich atherosclerotic plaque formation in the proximal aorta and aortic-tree vessels in a mouse model. Jain and colleagues[32] induced aortic lipid deposition in rabbits through a high fat-content diet while simultaneously inducing periodontitis in the mandibular molars in one experimental group. When compared with control animals in the second group without periodontitis, those animals with periodontal disease exhibited significantly greater accumulations of lipid (atheroma formation) in the aorta. Indeed, there was a positive correlation between the severity of periodontal disease and the extent of aortic lipid deposition.

Additionally, it has been demonstrated that whole cells of *P. gingivalis* and endotoxin derived from *P. gingivalis* are both capable of inducing, in vitro, foam-cell formation of mouse-derived macrophages when cultured in the presence of human low-density lipoprotein (LDL).[33] In another study, *P. gingivalis* and its endotoxin-laden vesicles promoted LDL binding to macrophages and promoted macrophage modification of native LDL, which plays an important role in foam-cell formation and the pathogenesis of atherosclerosis.[34] Further, Lalla and colleagues[35] demonstrated that mice, when infected with *P. gingivalis*, exhibited severe periodontitis, presence of *P. gingivalis* DNA in 22 percent of aortic biopsy specimens, and elevated serum IL-6 levels. Lastly, Gibson and colleagues[36] have shown that mice, when challenged with *P. gingivalis*, exhibited increased atherosclerotic plaque formation, which could be prevented by immunization against *P. gingivalis*.

Human studies —

Because of the purported roles of *P. gingivalis* and *A. actinomycetemcomitans* in severe chronic periodontitis, Pussinen and colleagues[37, 38] chose to analyze the association of coronary heart disease and ischemic stroke to antibody levels specific for these two microbes. They found that coronary disease was more prevalent among edentulous than dentate subjects (19.8 percent vs. 12.1 percent, respectively). Further, coronary disease was more common among patients with positive antibody levels (seropositive) for *P. gingivalis* as compared with those who were antibody-negative (seronegative) — 14 percent vs. 9.7 percent, respectively. Seropositive individuals had a risk ratio of 1.6 for an ischemic stroke event. In addition, subjects with a history of stroke or coronary heart disease were more often seropositive for *P. gingivalis* and had an risk ratio of 2.6 for a secondary stroke event. These results suggest that periodontal infections, or response of the host against such infections, may play a role in the pathogenesis of coronary heart disease and ischemic stroke.

Kuramitsu and colleagues[39, 40] studied the interaction of *P. gingivalis* with human umbilical vein endothelial cells and were able to show that *P. gingivalis* was capable of inducing increased expression of a cytokine — monocyte chemoattractant protein-1 — that recruits monocytes. In addition, *P. gingivalis* increases the expression of a protein that facilitates attachment of monocytes to endothelial cells, called intercellular adhesion molecule-1 (ICAM-1). Lastly, *P. gingivalis* increases the cellular production of elastase/gelatinase (MMP-9), which has been implicated in atheroma plaque rupture.[40] The authors hypothesize that *P. gingivalis*-endothelial cell interactions may lead to recruitment and attachment of monocytes to the endothelial lining of blood vessels, thereby initiating vascular atheroma formation.

Research on the relationship of inflammation to cardiovascular disease has begun to focus on heat shock protein 60 (HSP60), which is strongly immunogenic. Further, HSP60 appears to be a signaling molecule that can mediate and influence a range of inflammatory responses. For example, both bacterial and host HSP60 activate human vascular endothelial cell expression of intercellular and vascular cell adhesion molecules (ICAM-1 and VCAM-1). In addition, both types of HSP60 activate monocytes and/or macrophages to secrete IL-6 and TNF-α. Because of a high degree of sequence homology (molecular similarity) between bacterial and human HSP60, it has been suggested that HSP60 may be involved in human autoimmune disease mechanisms (i.e., the host immune system primed by HSP60 of bacterial origin can interact with its human host counterpart in gingival connective tissue or arterial walls).[41]

Yamazaki and colleagues[42] have examined the link between chronic periodontitis, atherosclerosis, and HSP60. Using both human and *P. gingivalis* HSP60 as the antigen, they compared humoral immune responses in atherosclerotic patients with responses in patients with

chronic periodontitis and in healthy patients. Results showed antibody levels to both human and *P. gingivalis* HSP60 were highest in atherosclerosis patients, followed by periodontitis patients, and lowest in healthy patients. Similar results have also been reported by Chung and colleagues.[43]

Desvarieux and colleagues[44] reported a direct relationship, independent of CRP levels, between thickness of the tunica intima and tunica media of the carotid artery (indicating atherosclerotic plaque formation) and the presence of five periodontal microbial pathogens, *P. gingivalis, A. actinomycetemcomitans, T. forsythia, Treponema denticola (T. denticola)*, and *Micromonas micros*. Shortly thereafter, Kozarov and colleagues[45] reported the presence of viable invasive *A. actinomycetemcomitans* and *P. gingivalis* in cells from human carotid artery atherosclerotic plaque. Marques da Silva and colleagues[46] used DNA probe techniques to examine 56 samples from aortic aneurysms taken from 51 patients for the presence of four periodontal microbial pathogens *(A. actinomycetemcomitans, P. gingivalis, T. denticola,* and *T. forsythia)*. They detected bacterial DNA in 89.2 percent of the specimens. However, *A. actinomycetemcomitans* was detected in only four specimens (7.1 percent), and all specimens were negative for the other three microbes. An explanation for this seeming paradox is found in a previous study by Marques da Silva and colleagues[47] in which anaerobic culture and electron microscopy techniques were used to demonstrate the presence of several common oral microbes, such as *Streptococcus mitis, Actinomyces naeslundii*, and *Actinomyces viscosus*.

Recently, Fiehn and colleagues[48] identified DNA from periodontal pathogenic microbes in atherosclerotic plaques that were removed from carotid and femoral arteries. DNA of *P. intermedia* was consistently detected, but *P. gingivalis* DNA was noted only sporadically. Interestingly, when cultured under anaerobic conditions, none of the tissue specimens yielded growth of oral bacteria. Additionally, Dögan and colleagues[49] compared the total bacterial number in subgingival plaque samples from periodontitis patients with and without a history of recent myocardial infarction. The authors reported that bacterial levels were elevated in only those patients with a history of myocardial infarction, leading the authors to suggest that increased loads of subgingival bacteria may present a risk for systemic health.

Current data does not indicate a direct involvement of the bacteria in development of aortic aneurysms. However, a dominant feature in the pathogenesis of aortic aneurysms is the proteolytic degradation of the aortic wall by MMPs. The expression of collagenase (MMP-1 and MMP-13) and elastase/gelatinase (MMP-2, MMP-9, and MMP-12) is increased in aortic aneurysm tissues. Theoretically, the presence of bacteria in a vascular wall lesion induces a localized inflammation with the inherent induction of various cytokines, primary mediators of inflammation that, in turn, stimulate MMP expression by host cells, eventually leading to an aortic aneurysm.

Clinical studies

Two studies in 1989 reported statistically significant relationships between oral health and myocardial and cerebral infarction.[50,51] Since that time, several studies have reported epidemiological associations between chronic periodontitis and cardiovascular and cerebrovascular disease.[4-8, 52-56] It is now obvious that periodontal disease and atherosclerotic plaque-related diseases have several risk factors in common, such as smoking, diabetes, elevated levels of serum CRP, etc. Because of overlapping risk factors, it remains difficult to demonstrate a direct causal relationship between chronic periodontitis and cardiovascular and cerebrovascular disease. However, this does not minimize the role of chronic periodontitis as an inflammatory risk factor in atherosclerosis and its sequelae.[10]

Many studies have examined the role of chronic periodontitis as an independent risk factor and an "infectious burden" in general; taken collectively, they indicate a significant association with atheroma formation (Figure 1) and ischemic stroke.[11,14,19,26,28,57-62] It has been suggested that periodontal inflammation may contribute to a prothrombotic state via recurrent bacteremias, platelet activation, and elevated clotting factors, thereby increasing the risk of embolism formation and ischemic stroke.[14]

Other studies suggest that it is highly unlikely that a single infectious agent or inflammatory disease plays a unique role in atheroma development. It is more likely that the risk of developing atherosclerosis is related to the number of inflammatory disease events to which an individual has been exposed.[61-63] One can argue that periodontitis represents a chronic inflammatory infection that may exist for years, thereby exposing the patient to a continuous microbial insult and all of the

inherent metabolic events associated with inflammation. Given such a scenario, moderate and severe chronic periodontitis represent an important risk factor for the development of atherosclerosis leading to cardiovascular and cerebrovascular disease. Thus, it is clinically relevant that three recent studies have reported that periodontal therapy consisting of scaling and root planing and subgingival delivery of antimicrobial agents is effective in reducing levels of serum inflammatory markers, specifically CRP, IL-6, and TNF-α.[64-66]

Conclusion

Inflammation in the vessel wall plays an essential role in the initiation and progression of atherosclerosis, the erosion or disruption of vascular atheromas, and eventual rupture of such plaques.[22] The collective body of literature suggests that immune activation in cases of severe chronic periodontitis results in the concomitant systemic dissemination of gram-negative microbes, antigens and endotoxins, and mediators of inflammation. The dissemination of these factors, in turn, appears to promote inflammation of the arteries involving the cardiovascular and cerebrovascular systems, leading to atherosclerosis, and ultimately initiating an acute coronary event or ischemic stroke, with circulating levels of the inflammatory markers reflecting the clinical course of the condition.

The general hypothesis that chronic infections, such as periodontitis, can contribute to the development of atherosclerosis and, thereby, cardiovascular disease and ischemic stroke, is based on the following observations:

- Infectious agents can directly interact with the cellular components of the tunica intima and tunica media of vessels.
- There is systemic dissemination of cytokines and mediators of inflammation because of chronic inflammatory disease, such as periodontitis.
- There is an increased expression of cytokines, mediators of inflammation, and cellular adhesion molecules resulting in local endothelial dysfunction.

Although the studies cited in this review point to a role for periodontal disease in the development of cardiovascular and cerebrovascular disease, it remains to be shown that treatment of periodontal disease will prevent atherosclerotic events. Currently, there is insufficient data to differentiate between the role of a direct infection of the vascular wall and stimulation of a proinflammatory state by periodontitis. In spite of these shortcomings, it is critical to test the hypothesis that intensive treatment of inflammatory periodontal disease and long-term maintenance will have a positive impact on the clinical course of atherosclerotic-related diseases.

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