An Update on Periodontal Infections, Systemic Inflammatory Biomarkers, and Cardiovascular Disease

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Cardiovascular disease (CVD) describes diseases of the heart and blood vessels, consisting mainly of coronary heart disease and stroke, which are among the main causes of premature death in humans. It is currently believed that long-lasting infections and low-grade chronic systemic inflammation play an important role in the pathogenesis of atherosclerosis and CVD. Periodontal diseases are induced by bacteria and bacterial products of plaque biofilm and characterised by inflammatory destruction of tooth-supporting connective tissues and alveolar bone. A number of case-control, cross-sectional and longitudinal studies indicate an association between periodontal disease and CVD after adjustment of common confounders, and a potential effect of periodontal infections on an increased risk of atherosclerosis, ischaemic heart disease and stroke, although a causal association of periodontal infection with atherosclerotic CVD remains to be elucidated. The emerging evidence suggests that periodontal disease, as one of the most common and unique infections in humans, may significantly contribute to systemic inflammation. Observations include the relationship between periodontitis and an elevated number of peripheral blood leukocytes, as well as increased levels of C-reactive protein (CRP) and IL-6, which may partly explain the association of periodontal disease with CVD, as documented in a number of studies. Although some initial studies have shown promising effects of periodontal treatment on the reduction of serum levels of CRP and IL-6, and improvement of the endothelial function in periodontitis patients, the potential mechanisms and exact effects of controlling periodontal infections on the reduction of systemic inflammation remain to be determined. Future studies are highly warranted to clarify these points and elaborate the relevant clinical implications.

Key words: atherogenesis, cardiovascular disease, C-reactive protein, inflammation, periodontal disease/infections

Periodontal infections are among the most common infections in humans and are characterised by bacteria-induced inflammatory destruction of tooth-supporting structures[1]. Gingivitis is the contained form of periodontal disease that manifests as redness and swelling of the gums, which bleed easily on stimulation, such as occurs during toothbrushing, while periodontitis presents with destruction of tooth-supporting tissues and alveolar bone[2]. In general, periodontal diseases are highly prevalent and can affect up to 90% of the worldwide population, and periodontitis remains the most common cause of tooth loss in adults[3,4].

Periodontal infections have been regarded as a source of focal infection for over 100 years[5]. In the last two decades, a growing body of scientific evidence has demonstrated an association of periodontal infections with cardiovascular disease (CVD). Currently, the pertinent question is about the nature and relevance of this as-

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This work was supported by the Hong Kong Research Grants Council (HKU 7518/05M) and The University of Hong Kong (CRCG 2006-2008).

sociation, i.e. either periodontal infections and the resultant inflammation contribute causally to CVD or these two clinical conditions just exist coincidently[6]. This literature review i) describes the nature and uniqueness of periodontal infections; ii) elaborates the potential biological mechanisms by which periodontal infections may affect the atherogenesis and contribute to CVD; and iii) updates current knowledge on selected systemic biomarkers of inflammation in relation to CVD, the contribution of periodontal infections to systemic inflammation, and the potential effect of periodontal treatment on systemic inflammatory biomarkers such as C-reactive protein (CRP) and IL-6.

Inflammation as a Key Pathogenetic Mechanism in Atherogenesis and CVD

CVD describes diseases of the heart and blood vessels, including mainly coronary heart disease (CHD) and stroke, which are among the main causes of premature death in the world. Previous studies demonstrate that the classical risk factors for the onset and progression of CVD[7], such as hypertension, hypercholesterolaemia and smoking, cannot account for all the variations in the incidence and severity of CVD. Conceivably, other unrecognised risk factors, such as some common chronic infections and inflammations, may play crucial roles[8,9]. Although infections have been suspected to be risk factors of CVD for 100 years, the hypothesis remained largely ignored until a herpes virus was shown to cause atherosclerosis in experimental animals[10-12]. In recent years, it has been appreciated that long-lasting infections and low-grade chronic systemic inflammation play an important role in the pathogenesis of atherosclerosis and CVD[8,13-18]. From a pathological viewpoint, the initiation, growth, and complication of the atherosclerotic plaque are considered to be an inflammatory response to injury. Virtually every step in atherogenesis is believed to involve cytokines, other bioactive molecules, and cells that are characteristic of inflammation, and these lead to the development of acute coronary and cerebrovascular syndromes[19]. Interestingly, infection and hypercholesterolaemia may have a synergistic effect[12].

This novel pathophysiological insight encourages the development of various diagnostic measurements and biomarkers for assessment and monitoring of the levels of systemic inflammation, including proinflammatory risk factors (e.g. oxidized low-density lipoproteins), proinflammatory cytokines (e.g. IL-1, TNF-α), adhesion molecules (e.g. intercellular adhesion molecule-1, selectins), inflammatory stimuli with hepatic effects (e.g. IL-6) or the products of the hepatic stimulation (e.g. serum amyloid A, CRP), and other acute-phase reactants (e.g. erythrocyte sedimentation rate), von Willebrand factor concentration as well as indicators of cellular responses to inflammation (e.g. elevated white blood cell count)[19,20]. The sources of the inflammation cascade have recently been reviewed, and include atherogenesis in coronary artery and other arteries, systemic inflammation (e.g. connective tissue diseases), and, notably, chronic, low-grade local infections/inflammations (e.g. gingivitis/periodontitis, prostatitis, bronchitis, urinary tract infections, and gastric inflammation)[19].

Nature of Periodontal Infections

Periodontal diseases are initiated and perpetuated by a group of predominantly gram-negative and anaerobic bacteria from dental plaque "biofilm"[21]. Periodontal diseases are among the most unusual human infections, due to the unusual anatomic feature of dento-gingival structure. In contrast to the outer surface of most parts of the body, the outer layers of the tooth provide a relatively stable surface for microbial colonisation, allowing microorganisms to remain in immediate proximity to the periodontal tissues. Furthermore, the non-shedding tooth surface and the attached microorganisms are immersed in an aqueous environment, where the bacterial infection can be less controlled by the potent mechanisms of host defence and antimicrobial therapy[22]. It is therefore conceivable that the plaque biofilm is extraordinarily persistent and difficult to eliminate without professional care. The altered subgingival environment allows for the establishment of a complex subgingival plaque with many potentially pathogenic microorganisms. The bacteria and their toxins irritate the periodontal connective tissues and stimulate a chronic immunoinflammatory response. The severity of the resultant periodontal destruction is dependent upon dynamic interactions between the microbial challenge and the host immuno-inflammatory response, which are influenced by a series of risk factors, including systemic factors (e.g. diabetes, immunodeficiency diseases, stress and osteoporosis), genetic variations, behavioural and environmental factors (e.g. tobacco smoking)[23-28].

In advanced periodontitis, the thin, highly permeable and frequently ulcerated deep pocket epithelium is the only barrier between the bacterial biofilm and the underlying connective tissue. The strands of the pocket epithelium are easily broached, allowing large doses of bacterial toxins and other products access to the tooth-supporting connective tissues and blood vessels. It has been estimated that in a relatively complete dentition with ad-

vanced periodontitis, the total area of infected and inflamed pocket epithelium is about 72 cm^2 – the size of the palm of the human hand[29]. It is apparent that an uncontrolled severe periodontitis presents a substantial infectious and inflammatory burden for systemic health, and the periodontal pocket and the adjacent inflamed periodontal tissues may serve as a renewing reservoir for spillover of bacterial antigens, bacterial products and inflammatory mediators[25,30] into the circulation, which may subsequently affect the vascular endothelium and other cells and tissues distant from the periodontal tissues[29].

Periodontal Infections and CVD

Periodontal disease, being a common infection, has undergone substantial research to evaluate its relation with atherogenesis and CVD. In the last 15 years, a series of case-control, cross-sectional studies, and longitudinal studies on the association between periodontal disease and systemic inflammation as well as CVD have been documented by various groups of researchers around the world[6,31]. These case-control and cohort studies show significant positive associations between periodontal disease and CVD, with odds ratios (OR) ranging from 1.3 to 14.1, and mostly under 2.0. Similarly, the cross-sectional studies suggest significant positive associations, after adjusting confounding factors such as age, sex, diabetes, cholesterol levels, blood pressure, obesity, smoking status, dietary patterns, ethnicity, educational background and socioeconomic status[6,31]. Most of the well-documented longitudinal studies, currently the best form of evidence available, indicate an association between periodontal diseases and CVD, and potential effects of periodontal infections on an increased risk of atherosclerosis, ischaemic heart disease and stroke. However, a causal association of periodontal infection with atherosclerotic CVD remains to be elucidated, particularly concerning the complexity of interactions among common risk factors, exposures used for assessment of periodontal disease and infections, and outcome measures of CVD[31]. A meta-analysis study showed that periodontal disease contributed to a 19% increase in risk for future CVD in all age groups and a 44% increase of risk in subjects aged 65 years or less[32]. Furthermore, studies on periodontal disease and stroke show relatively stronger relationships than those on periodontal disease and CHD, and most of them demonstrate that periodontal infection is significantly associated with ischaemic and haemorrhagic stroke[31]. Our recent 16-year follow-up study suggests that experience of periodontitis with missing molars in young adults might be associated with certain life-threatening diseases (e.g. circulatory disease) that contribute to premature death[33]. Taken together, most of the studies suggest an association of CHDs and stroke with periodontal disease, measured both clinically and radiographically. However, several null reports give good reasons for caution regarding the potential limitations of these studies, such as the non-specificity of clinically and radiographically defined exposures of periodontal infections used by these traditional studies[6]. Further studies are warranted to target direct assessment of periodontal infection exposure and the resultant systemic inflammation in relation to appropriate measurement of subclinical status of CVD and related biomarkers.

In recent years, novel research has refined the study protocols to focus more directly on the interactions of infectious and inflammatory periodontal disease processes with appropriate outcome measures of atherogenesis and subclinical CVD. Peripheral vascular disease and CVD may share atherosclerosis as a common pathway. A previous study reported that periodontal disease might be a significant independent risk factor for development of peripheral vascular disease[34]. A study with over 6,000 adults found that severe periodontitis was related to increased intima–media wall thickness, with an OR of 1.31 after adjustment for various confounders[35]. A recent study suggested that periodontitis might be associated with the development of early atherosclerotic carotid lesions[36]. A series of recent studies showed that increased systemic antibodies to periodontopathogens were significantly associated with atherosclerosis, CHD and risk of CVD progression[37-41]. Carotid intima-media wall thickness, a measure of subclinical atherosclerosis, increased with levels of the periodontopathogens[42]. The results suggest a possible role of periodontal infection in the pathogenesis of atheroma formation[35,43]. Recently, Spahr and coworkers[44] showed the association of increased periodontopathogen burden with the clinical condition of CHD.

Biological Mechanisms linking Periodontal Infections and CVD

Three pathways have been described to link the oral/periodontal infections with systemic inflammation[45]: i) metastatic infection caused by translocation of bacteria; ii) metastatic injury related to microbial toxins; and iii) metastatic inflammation due to immune injury. However, the exact biological mechanisms by which periodontal infection may affect atherogenesis and contribute to CVD are not fully understood[26]. It is speculated that the inflamed and ulcerated pocket epi-

thelium may form an easy port of systemic access to circulation system for Gram-negative and anaerobic bacteria. Bacterial components, such as major outer membrane proteins and endotoxins (e.g. lipopolysaccharides, LPS), may be disseminated in blood circulation[46]. It is known that poor oral hygiene and periodontal or periapical infections may produce bacteraemias even in the absence of dental procedures[47].

Bacteraemias may be intensified in patients with periodontitis[48]. It has been shown that the incidence and magnitude of bacteraemias of oral origin are directly proportional to the degree of oral inflammation and infection[49,50]. The levels of induced bacteraemia following chewing action and periodontal instrumentation increased more significantly in periodontitis patients compared with normal controls or gingivitis subjects[46,51]. In response to the bacteraemia and bacterial antigens that are systemically dispersed, blood and tissue cells at locations where the antigens are relocated may produce cytokines and pro-inflammatory mediators, thereby contributing to the systemic inflammation and CVD.

Periodontal pathogens have also been identified in atheromatous plaques[52,53], and actively invade human endothelial cells[54] as well as vascular endothelium[52,55]. Direct invasion of vessel walls by oral pathogens could trigger an inflammatory response that may translate into endothelial dysfunction[56]. *In vitro* and animal studies showed that periodontopathogens could enhance platelet aggregation and induce the formation of foam cells[57,58]. Recent studies showed that *P. gingivalis*, a key periodontopathogen, may play a crucial role in the initiation and exacerbation of atherosclerosis[56,59-61].

Furthermore, the locally produced pro-inflammatory mediators in inflamed periodontal tissues, such as IL-1, TNF-α, IL-6, and PGE_2, may be "dumped" into the circulation and exert systemic or distant effects[62-64], contributing significantly to chronic, systemic vascular challenge, and directly results in platelet aggregation, adhesion and vasculitis, and the subsequent cholesterol deposition, thromboembolic events and atheroma formation[26,29]. Another plausible mechanism is that the inflammation caused by periodontal disease induces inflammatory cell infiltration into major vessels, vascular smooth muscle proliferation, vascular fatty degeneration, and increased plaque build up, which contribute to swelling and thickening of the arteries[65]. These events may lead to atherosclerosis and atheroma formation, and result in obstruction of normal blood flow, restricting the amount of nutrients and oxygen required for the heart to function properly, and eventually increase the risk of heart attacks[2,66]. In addition, CVD and periodontal diseases have a number of common characteristics and may share a similar causative pathway through a hyper-inflammatory phenotype, e.g. increased release of inflammatory cytokines and relevant mediators[26].

Association of Periodontal Infection with Systemic Inflammation

Since atherogenesis may in part be an inflammatory disease, and circulating inflammatory biomarkers like CRP may be predictors of CVD[15,67], the changes of these inflammatory biomarkers in peripheral blood in periodontitis may explain the underlying mechanisms that support the epidemiological observations on the association of periodontitis with atherogenesis, thromboembolic events and CVD[64]. In recent years, one of the research interests in periodontal medicine has focused on periodontal infections as a potential contributor to systemic inflammation, which is usually measured with count of cellular components and serum levels of selected inflammatory biomarkers, such as CRP, fibrinogen, and pro-inflammatory cytokines[64,68]. Of the circulating inflammatory biomarkers, CRP has been the focus of research in recent years. CRP has proved to be the strongest and most significant predictor of the risk of future cardiovascular events among several plasma variables[69,70]. A recent statement from the Centers for Disease Control and Prevention and the American Heart Association concluded that CRP as a sensitive circulating marker of inflammation may serve as an adjunct to the measurement of established risk factors of CHD, and that three risk categories of subjects could be classified according to serum levels of CRP: low risk, < 1 mg/l; medium risk, 1–3 mg/l; and high risk, > 3 mg/l[19].

It has been shown that periodontitis is related to elevated numbers of peripheral blood leukocytes[64,71], lower numbers of erythrocytes and lower levels of haemoglobin, so called 'anaemia of chronic disease'[72]. These serum inflammatory biomarkers, especially CRP, have been studied intensively as possible markers of systemic inflammation involved in periodontal infections[64,68]. The studies showed that plasma or serum levels of CRP[63,73-79] and IL-6[63,77,80] were consistently higher in periodontitis patients than in healthy controls. Both CRP[63,77,78,81,82] and IL-6[63,80] were related to the severity and extent of periodontitis. The latter was thought to activate hepatocytes to produce acute-phase reactants like CRP[83].

Although periodontal disease is associated with increased serum inflammatory biomarkers like CRP and IL-6, the effect of periodontal treatment on these markers remains to be determined. Recent studies showed that

control of periodontal infection through non-surgical treatment could reduce serum levels of CRP and IL-6[73,82,84-87]. Periodontal treatment could also improve the endothelial dysfunction in patients with severe periodontitis, accompanied by a significant decrease in CRP concentrations[88]. There is increasing evidence from clinical trials that the improvement of endothelial function may translate into lower rates of cardiovascular events[89]. In addition, intensive periodontal therapy could reduce the levels of total and LDL cholesterol[90]. A recent systematic review and meta-analysis on the effect of periodontal treatment on serum CRP levels show that unusual CRP levels could be significantly modulated by a single episode of non-surgical periodontal therapy in patients with severe periodontitis[68]. Considering the individual variation of CRP response to periodontal therapy, a recent study investigated the effect of a CRP polymorphism (+1444C>T) on the CRP levels in response to periodontal therapy[90]. The results showed that the individuals homozygous for the +1444T-allele exhibited higher CRP concentrations than C-allele carriers, independent of conventional cardiovascular risk factors and inflammatory factors known to affect CRP levels. Therefore CRP genotype should be considered when studies on CRP are performed. Further well-designed, randomly controlled trials with an appropriate treatment protocol are warranted to clarify whether effective control of periodontal infections could contribute to positive modulation of systemic inflammation.

Summary

It is currently believed that chronic, low-grade and long-lasting infections and inflammation play a major role in the pathogenesis of atherogenesis and CVD. Of the circulating inflammatory biomarkers, CRP has proved to be one of the most significant predictors of the risk of CVD among the plasma variables. The emerging evidence shows that periodontal disease, as one of the most common infections in humans, may significantly contribute to systemic inflammation. Observations include a relationship between periodontitis and elevated numbers of peripheral blood leukocytes as well as increased levels of CRP and IL-6, which may partly explain the association between periodontal disease and CVD, as documented in a number of case-control, cross-sectional and longitudinal studies. However, a causal association of periodontal infection with atherosclerotic CVD remains unclear. Although some initial studies have shown promising effects of periodontal treatment on the reduction of serum levels of CRP and IL-6, and improvement of the endothelial function in periodontitis patients, the potential mechanisms and exact effects of controlling periodontal infections on the reduction of systemic inflammation remain to be determined. Further studies are highly warranted to clarify these points and elaborate the relevant clinical implications.

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