CONCISE REVIEW

*International Dental Journal* 2015; 65: 283–291

doi: 10.1111/idj.12185

# Systemic effects of periodontitis: lessons learned from research on atherosclerotic vascular disease and adverse pregnancy outcomes

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Studies conducted over the past 25 years have focussed on the role of periodontitis, an inflammatory condition of microbial aetiology that destroys the tooth-supporting tissues, as a systemic inflammatory stressor that can act as an independent risk factor of atherosclerotic vascular disease (AVSD) and adverse pregnancy outcomes (APOs). It has been suggested that periodontitis-associated bacteraemias and systemic dissemination of inflammatory mediators produced in the periodontal tissues may result in systemic inflammation and endothelial dysfunction, and that bacteria of oral origin may translocate into the feto-placental unit. Epidemiological studies largely support an association between periodontitis and ASVD/APOs, independently of known confounders; indeed, periodontitis has been shown to confer statistically significantly elevated risk for clinical events associated with ASVD and APOs in multivariable adjustments. On the other hand, intervention studies demonstrate that although periodontal therapy reduces systemic inflammation and improves endothelial function, it has no positive effect on the incidence of APOs. Studies of the effects of periodontal interventions on ASVD-related clinical events are lacking. This review summarises key findings from mechanistic, association and intervention studies and attempts to reconcile the seemingly contradictory evidence that originates from different lines of investigation.

***Key words:*** *Inflammation, periodontal, cardiovascular, gestation, therapy, review*

## INTRODUCTION

Inflammation is at the heart of several pathological conditions that have been traditionally considered as non-inflammatory. Consequently, over the last 25 years, a large body of research has focussed on the potential effects of periodontitis, a bacterially driven inflammatory disease, on general health outcomes. After the early publications by Mattila *et al.*[1], on the association between poor dental health and myocardial infarction, and by Offenbacher *et al.*[2], on the possible link between periodontitis and preterm low birth weight, a new field of periodontal research has emerged, collectively referred to as 'periodontal medicine'. This article provides a concise overview of the association between periodontitis as a potential exposure conferring risk for two broadly defined extra-oral pathologies, namely atherosclerotic vascular disease (ASVD) and adverse pregnancy outcomes (APOs). The text will review aspects of biological plausibility, will summarise the key epidemiological findings and will offer thoughts about the appropriate interpretation of the currently available research data.

## BIOLOGICAL PLAUSIBILITY

Periodontal diseases are inflammatory disorders that are the product of a polymicrobial perturbation of host homeostasis in the soft and hard tissues that support the teeth[3]. Epidemiological data suggest that their prevalence is high, both in the USA[4] and globally[5]; therefore, a potential role of these reversible and treatable conditions, as systemic inflammatory stressors that may confer risk for extra-oral pathology, is critically important from a public health point of view. Periodontitis is characterised by loss of connective tissue attachment and alveolar bone and is accompanied by conversion of the shallow gingival

sulcus into a deep periodontal pocket and a marked proliferation of a dysbiotic subgingival biofilm[6]. In cases of generalised periodontitis, the surface area of the epithelial lining of the periodontal pocket is substantial[7], and breach of this epithelial barrier through ulceration allows direct contact between the microbial biofilm and the connective tissue. At the same time, this pocket epithelium/biofilm interface provides a gate of entry through which bacteria and bacterial products, including lipopolysaccharide (LPS), challenge the immune system, elicit a local inflammatory response[8] and also enter the circulation. Indeed, transient bacteraemias occur frequently as a result of daily activities such as toothbrushing or chewing[9,10], as well as during invasive oral therapeutic procedures[11,12]. In parallel, inflammatory mediators that are abundantly produced locally in the periodontal tissues[13], including pro-inflammatory cytokines, chemokines and (as recently demonstrated) gingiva-derived C-reactive protein (CRP)[14], can also be disseminated systemically through the bloodstream. A systemic acute-phase response, subsequent to processing of antigenic structures by the liver, results in elevated plasma CRP in patients with periodontitis. Indeed, a meta-analysis of cross-sectional studies of high-sensitivity CRP (hs-CRP) plasma levels in patients with periodontitis, compared with those of periodontally healthy controls, demonstrated a statistically significantly higher mean weighted hs-CRP plasma levels in patients with periodontitis, of 1.56 mg/L. This difference can be biologically relevant, as it can potentially shift an individual to the high CRP risk category (>3 mg/L)[15].

As recently reviewed[16,17], vascular endothelial activation is central to the role of periodontal infection/inflammation as a risk factor for atherosclerosis, but also as an exposure of importance for pre-eclampsia, a pathological condition that occurs during the second trimester of pregnancy and is characterised by maternal hypertension and proteinuria. In brief, circulating bacterial products, such as lipopolysaccharide (LPS), outer membrane vesicles and fimbriae, or inflammatory cytokines and chemokines, result in an up-regulation of cell-surface receptors and in expression of adhesion molecules on the endothelial lining of the vasculature. As a result, peripheral blood monocytes are recruited and adhere to the activated endothelium. 'Molecular mimicry'[18] (i.e. the process through which antibodies targeted against specific bacterial proteins – for example, 'heat-shock' proteins that are highly homologous with mammalian proteins – act as autoantibodies) contributes to apoptotic damage of the vascular endothelium. Guided by a chemoattractant gradient, monocytes migrate into the subendothelial space, transform into tissue macrophages, uptake oxidised low-density lipoprotein (LDL) cholesterol and become foam cells. Apoptosis of LDL cholesterol-laden macrophages results in accumulation of lipids in the subendothelial space (i.e. in the formation of atheromatic plaques, which are covered by a fibrous cap and facilitate platelet accumulation). Exposure of the fibrous cap after endothelial cell apoptosis and enzymatic degradation of the extracellular matrix results in plaque rupture, exposure of prothrombotic plaque components and subsequent thrombus formation, which leads to the occlusion of the vessel. This may be manifested as a clinical ASVD-related event (e.g. a myocardial infarction in the case of an occluded coronary artery, or a stroke in the case of an occluded cerebral vessel).

Interestingly, experimental mechanistic studies using oral inoculation with the keystone periodontal pathogen, *Porphyromonas gingivalis*, showed accelerated atherosclerosis in apolipoprotein-E null mice, and localisation of *P. gingivalis* DNA in the aortic wall[19]. The latter observation is in agreement with findings from human endarterectomy specimens that showed the presence of DNA from several periodontal species[20], and with observations of recovered viable *Aggregatibacter actinomycetemcomitans* and *P. gingivalis* from human atheromatic plaques[21].

With respect to the plausibility of the association between periodontitis and selected APOs (i.e. preterm birth, low birth weight and pre-eclampsia), haematogenous spread of periodontal bacteria and their products, and subsequent seeding of the placental membranes, has been proposed as one of the potential mechanisms[22]. Notably, a number of maternal infections, including uterine tract infections, bacterial vaginosis and chorioamnionitis, are established risk factors for preterm birth and low birth weight[23], although approximately 50% of the variance in the incidence of these conditions remains unexplained by the established risk factors[24].

Normal parturition is controlled by inflammatory signalling that can be modified by external stimuli, including infection and inflammatory stressors[25]. As pregnancy progresses, the amniotic fluid levels of prostaglandin $E_2$ ($PGE_2$) and inflammatory cytokines, such as tumour necrosis factor alpha (TNF-$\alpha$) and interleukin-1beta (IL-1$\beta$) rise steadily until a critical threshold level is reached, at which point they induce rupture of the amniotic sac membranes, uterine contraction, cervical dilation and delivery. Thus, elevation of these mediators in the amniotic sac walls or the myometrium may precipitate a premature onset of parturition. As mentioned above, the stimulus for this inflammatory response can reside either in the vicinity of the feto-placental unit or it may originate from more remote loci.

Early evidence of the potential role of translocated oral bacteria in APOs was provided by Hill[26], who

reported that amniotic fluid cultures from women with vaginosis frequently harboured *Fusobacteria* of oral origin. These observations were extended by Han *et al.* [27], who demonstrated transmission of an uncultivated *Bergeyella* strain from the oral cavity to the amniotic fluid in a case of preterm birth. Experimental evidence in the pregnant hamster[28,29] showed that injection of *P. gingivalis* resulted in intrauterine growth retardation. Subsequent studies in pregnant mice and rabbits[30,31] confirmed and expanded these observations to include experimental infections with *Fusobacterium nucleatum* and *Campylobacter rectus*.

## EPIDEMIOLOGICAL EVIDENCE

Before summarising the key findings from studies in humans on the role of periodontitis as a risk factor for ASDV or APOs, it is important to emphasise three key issues.

The first issue is that the exposure variable, in our case periodontitis as a potential risk factor, has been defined in epidemiological studies using a variety of measures, including clinical parameters (probing depths, attachment levels and bleeding on probing), radiographic assessments of alveolar bone loss, self-reported periodontal status, surrogate markers of poor oral health (tooth loss or edentulism), subgingival microbial profiles or (in some sero-epidemiological studies of ASVD) levels of antibodies to periodontal bacteria. It is clear that these variables are not interchangeable, as they reflect different aspects and phases of the disease pathobiology, and in the case of edentulism, a much more complex interplay of oral morbidity, socio-economic factors, access to care and therapeutic decisions. The second issue is that even studies which have assessed periodontal status by clinical means in a comprehensive manner have not employed a consistent definition of periodontitis, but have used variable composite constellations of gingival inflammation, pocket depths and attachment levels to define a 'periodontitis case'. The third point, one of the key determinants of quality in an epidemiological study, is whether the association between the exposure under investigation (i.e. periodontitis) and the outcome (i.e. ASVD and APOs) has been adjusted for additional exposures that are known to affect the outcome (e.g. age, hyperlipidaemia, hypertension and physical activity), as well as for potential confounders [i.e. factors that are associated with both the exposure under study (periodontitis) and the outcomes (ASVD or APOs) such as smoking and/or race/ethnicity]. Therefore, it must be recognised that both the differences in exposure definitions across studies and the variable degree of adjustment for additional risk factors may underlie a large proportion of the non-concordant findings in the literature.

### Periodontal disease and ASVD

The available epidemiological studies between periodontitis and ASVD can be grouped in three major categories: (i) association studies (cross-sectional, case–control or longitudinal cohort studies) focussing on surrogate markers of ASVD; (ii) association studies focussing on clinical events (i.e. coronary heart disease, myocardial infarction, cerebrovascular disease and peripheral artery disease); and (iii) intervention studies, examining the effects of periodontal therapy on AVD-related outcomes (surrogate markers or events).

The first group of studies has typically examined measures of systemic inflammation, such as CRP[32–34], or coagulation markers associated with clinical events, such as fibrinogen[35,36], and have generally reported higher levels of these mediators in patients with periodontitis than in periodontally healthy controls. As mentioned above, meta-analysis of observation studies demonstrated significantly higher levels of hsCRP in periodontitis than in periodontal health[37]. Another marker of subclinical atherosclerosis that has been studied in this context, and is known to be associated with increased risk or myocardial infarction and stroke, is intima-media thickness (IMT)[38]. Beck *et al.*[39] analysed cross-sectional data on 6,017 participants in the Atherosclerosis Risk in Communities (ARIC) study, and demonstrated that severe periodontitis conferred risk for increased IMT. Findings from the Oral Infection and Vascular Disease Epidemiology Study (INVEST) demonstrated, in a fully adjusted model, that IMT and white blood cell counts increased significantly over tertiles of colonisation with specific periodontal bacteria; this is suggestive of bacterial dysbiosis[40]. A similar association was observed between subgingival microbial dysbiosis and both diastolic and systolic blood pressure, as well as prevalent hypertension[41]. Interestingly, serum antibody levels to several periodontal species were found to be positively associated with both prevalent IMT[42] and incident IMT progression[43]. Lastly, the latest report from the INVEST study, based on 430 participants followed up over a median time of 3 years, demonstrated that progression of carotid IMT varied inversely across quartiles of longitudinal improvement in clinical and microbial periodontal status[44].

A large number of studies published over the last two decades have examined the association of poor periodontal status and clinical ASVD-related outcomes, including coronary heart disease, myocardial infarction and stroke. Despite a substantial variability in the findings (expressed through odds ratios, hazard ratios and relative risk estimates), many – but clearly not all – publications report statistically significant associations after adjustment for covariates and

potential confounders. At least three meta-analyses that summarised the association between periodontal disease and clinical cardiovascular outcomes[45–47] consistently concluded that the available evidence suggests a moderate, positive association. This conclusion was corroborated by two recent narrative reviews[16,48]. A recent meta-analysis, focussed exclusively on cerebrovascular outcomes, reported a positive association between periodontitis and both ischaemic and haemorrhagic stroke[49]. Interestingly, the effect of periodontitis on ASVD-related clinical outcomes appears to be differential with age, with stronger associations in younger adults (<60 years of age) than in older adults[50,51]. In addition, studies that have reported significant positive associations among never-smoking participants[52–54] suggest that it is unlikely that the observed associations can be solely attributed to residual confounding as a result of the effects of smoking[55].

The third category of studies consists of intervention studies that examine the effect of periodontal therapy on ASVD-related outcomes. The ideal design for these studies is that of a randomised, controlled clinical trial (RCT) that provides the highest level of evidence and minimises bias. In the case of periodontitis/AVSD association, however, the conduct of such studies is particularly challenging, given the relatively low incidence of ASVD-related events in the population that necessitates enrollment of large population samples, the extended time period between the onset of subclinical atherosclerosis and the manifestation of the clinical event and ethical considerations related to the follow up of untreated periodontal disease over prolonged time periods. Therefore, apart from a single pilot feasibility secondary prevention study of cardiac events (the Periodontitis and Vascular Events study[56,57]), all other intervention trials conducted to date have primarily focussed on assessing the short-term effects of periodontal therapy on surrogate markers of AVSD, such as inflammatory mediators in the peripheral blood[58–61]. The most recent meta-analysis of RCTs, evaluating the effect of periodontal therapy on the reduction of systemic inflammation[62], included a total of 2,561 randomised patients and showed that periodontal therapy resulted in a significantly higher mean reduction in plasma CRP than the control arm, of 0.37 mg/L ($P = 0.005$). Interestingly, a trend for stronger treatment effects was noted in patients with comorbidities than in systemically healthy patients, although the interaction was not significant. Three small-sized, single-arm intervention studies reported positive effects of periodontal therapy on endothelial dysfunction, a marker of vascular disease[63]. Of these, one study employed mechanical, non-surgical periodontal therapy[64], another used mechanical therapy accompanied by adjunctive systemic antibiotics[65], whilst the third[66] carried out scaling and root planing and, when indicated, periodontal surgery and tooth extractions within a 2-week period. A larger RCT, involving a total of 120 patients with severe periodontitis, half of whom received full-mouth subgingival debridement completed within a single session and accompanied by topical application of minocycline in all deep periodontal pockets[67], demonstrated a significant improvement in endothelium-dependent dilatation (EDD) in the treatment group at a 6-month follow-up examination.

Lastly, a small single-arm study showed that non-surgical periodontal therapy results in diminished IMT thickness at 6 and 12 months[68].

## Periodontal disease and APOs

The first case–control study of pregnant or postpartum women, published in the mid-1990s, showed that women giving birth to preterm, low birth-weight babies had significantly higher mean clinical attachment loss than did mothers with full-term, normal birth-weight babies[2]. The most recent systematic review of observational studies available to date[69] screened approximately 700 articles, using predefined criteria, to evaluate the potential role of maternal periodontitis during gestation and three primary pregnancy complications (preterm birth, low birth weight and pre-eclampsia). The authors discussed, in detail, a number of features related to the methodology used and the overall quality of the studies, all of which could conceivably impact the strength of the association under investigation. These features included the type of periodontal examination performed (whether it was based on a full-mouth or partial-mouth examination); the consistency in the timing of the examination with respect to gestational age (antepartum or postpartum); involvement of examiners who were 'blinded' to the outcome; and whether multivariable analyses, including known or suspected exposures of significance and confounders, were carried out. The review also analysed the impact of using continuous (e.g. mean pocket depth and mean attachment level) *versus* categorical (presence/absence) definitions of periodontitis, especially given the absence of a universally accepted definition of periodontitis in general, and in particular in younger age groups, such as women of childbearing age. Indeed, more than 50 continuous parameters and 14 different definitions of cases (not specific to pregnancy) have been used in these studies[70].

Despite the inevitable inconsistency in the literature findings as a result of both true variability in the association between periodontitis and APOs in different populations, and the methodological aspects described

above, the systematic review concluded that there is a positive association between poor maternal periodontal status and all three adverse pregnancy outcomes examined (preterm birth, low birth weight and pre-eclampsia). With respect to the magnitude of the effect, the review identified it as modest, but independent of other exposures (i.e. not solely attributed to other risk factors that, when present in women of childbearing age, contribute to APOs, such as poor socio-economic status, young maternal age, certain race/ethnicity characteristics and cigarette smoking). Thus, the epidemiological evidence from the association studies available so far is largely consistent with the biologically plausible role of periodontitis as a systemic stressor that was described above.

At least 13 RCTs have been published so far examining whether treatment of maternal periodontitis during the second trimester may lower incident preterm birth or low birth weight, collectively enrolling more than 7,000 pregnant women with periodontitis or gingivitis. Eight RCTs contributed with at least 300 participants each[71–78] and two[75,76] with over 1,000 participants each, arguably rendering the assessment of the effects of periodontal interventions on APOs the most thoroughly investigated research topic in dental medicine. In these trials, approximately half of the women were randomised to receive mechanical periodontal therapy (accompanied by adjunctive antiseptic rinsing or systemic antibiotics in a number of studies) before completion of the second trimester. Synthesis of the findings of these studies in systematic and narrative reviews[79–81] concluded that nonsurgical treatment of periodontitis during the second trimester does not result in decreased rates of preterm delivery and does not have a positive impact on birth weight.

## WHAT SHOULD WE MAKE OF IT ALL?

So where do we stand after 25 years of intense research and tens, if not hundreds, of millions of dollars spent worldwide to give an answer to the question of whether periodontitis causes systemic disease? In the opinion of this author, if the question had been phrased in a more sensible manner, the answers would have been easier to disseminate to both our patients and the public alike. Let me explain: complex pathological conditions such as atherosclerosis and adverse pregnancy outcomes (and indeed also periodontitis) are a result of the interaction of multiple factors that are collectively responsible. With the notable exception of genetically conferred Mendelian diseases, in which the function of a single gene is solely responsible for the occurrence of the pathological phenotype, what is 'the' causative exposure is the wrong question to ask, simply because there is no single causative factor that can be identified. Instead, the aetiology of a complex disease encompasses the aggregate effects of multiple exposures, external and host-derived, detrimental and protective, each of which can account for only a fraction of the variance in the outcome in the population. Obviously, some exposures are of more decisive importance than others, or may be commonly present in a larger proportion of those affected. However, they are very seldom both the *necessary and sufficient* condition for disease to occur. To give an analogy from the cardiovascular research world, hypertension, poor diet, smoking and sedentary lifestyle are not 'causative' factors for myocardial infarction or stroke, although they confer increased risk for the development of these conditions. In other words, strokes do still occur among normotensive individuals, and many sedentary smokers with poor dietary habits will never experience a myocardial infarction. Therefore, instead of asking whether periodontitis causes ASVD or APOs, it is more reasonable to assess whether periodontitis, and all the pathophysiological sequalae that accompany this oral disease, are likely to contribute towards or against the development of these conditions. Viewed collectively, the preponderance of evidence from both mechanistic studies and human epidemiological data corroborate the view that periodontitis acts as a biologically plausible risk factor. Indeed, after reviewing the available evidence, the scientific statement of the American Heart Association clearly stated that 'observational studies to date support an association between periodontitis and ASVD independent of known confounders'[48]. Although the possibility that so far *unrecognised* confounders (for example, genetic predispositions that affect both periodontitis and ASVD) cannot be ruled out, the current interpretation is that periodontitis constitutes a systemic inflammatory stressor that contributes to increased risk for ASVD. Unfortunately, instead of concentrating on this important message, disproportionate attention of the public and the press focussed on a subsequent statement of the same publication that read 'They do not, however, support a causal relationship', which is a less meaningful proposition for the reasons explained above. Furthermore, the report's statement 'Although periodontal interventions result in a reduction in systemic inflammation and endothelial dysfunction in short term studies, there is no evidence that they prevent ASVD or modify its outcomes' has also been largely misinterpreted by the media and the public: as discussed in a commentary by two co-authors of the AHA Scientific Statement[82], the quoted lack of evidence reflects the fact that the appropriate studies (i.e. RCTs examining the effect of periodontal interventions on ASVD-related clinical events) have not been conducted. It does not imply that there is evidence demonstrating that periodontal interventions are ineffective in preventing/ameliorating ASVD.

The state of the science is quite different on the issue of periodontitis as an independent risk factor for APOs: while a synthesis of the available epidemiological data concludes that 'maternal periodontitis is modestly but significantly associated with preterm birth, low birth weight and preeclampsia'[69], high-quality intervention studies have been conducted, and their outcomes clearly do not support the notion that treatment of maternal periodontal disease in the second trimester may improve gestational outcomes. So, how can we reconcile the non-concordant data from the observational and the intervention studies? Before attempting to do this, it is important to recap on what the purpose of clinical trials is and how their findings should be correctly interpreted. RCTs are designed to inform public health policy and to guide the best clinical-practice approaches. They test, under very rigorous conditions, a very specific intervention scheme and its effects on a prespecified outcome. If the results of an RCT indicate that the tested intervention is indeed effective in improving the prespecified outcome, then the findings, apart from demonstrating the effectiveness of the intervention, also point to a factor that is involved in the causal pathway for the disease under study. Interpretation of negative RCTs is immensely more complicated: if corroborated by additional trials, the findings can establish that the tested intervention cannot improve the outcome under investigation. This is, however, not necessarily synonymous with the conclusion that the factor targeted by the intervention is unrelated to the disease. There are multiple reasons why interventions targeting a true causative factor may still fail: these include (i) ineffective intervention; (ii) inappropriate timing of the intervention; or (iii) irreversible damage caused by the factor before the onset of the intervention. In other words, in the case of periodontitis and APOs, it is still possible that the RCTs failed to demonstrate a positive effect of periodontal therapy, not because periodontitis is unrelated to APOs but because the treatment failed to suppress periodontitis beyond a critical level required for the beneficial effect to be manifested, or the treatment was rendered at an inappropriate time point. In addition, given that periodontal therapy results in a short-term increase in systemic inflammation and deterioration of endothelial function owing to massive inoculation with bacterial antigens during scaling and root planing, it is reasonable to speculate that the limited time between the intervention (the end of the second trimester) and delivery does not allow the manifestation of any associated benefit, such as that realised in terms of endothelial function 6 months after periodontal therapy[67]. In other words, it is possible that treatment of maternal periodontitis in the preconception period might have yielded entirely different results. From a public health point of view, however, the question of whether maternal periodontal therapy provided during gestation results in improved pregnancy outcomes is settled, and the answer is no. Neither is it particularly meaningful to speculate on what would have happened if all treated women had ended up with plaque levels and gingival indices close to zero (i.e. if interventions resulted in much better periodontal outcomes). As treatment of several thousand women demonstrated that this is not readily achievable, the issue of the utility of appending periodontal therapy to the prenatal care package of pregnant women for the purpose of improving pregnancy outcomes has also been definitively addressed. By no means, however, can the data from the negative RCTs be used as proof that maternal periodontitis is unrelated to preterm/low birth weight. Answer to this question requires the collective assessment of the evidence from multiple sources of investigation, not solely the results from intervention trials.

It would be unfortunate if the misinterpretations of the current state of evidence discussed above, in combination with the understandable reluctance of funding agencies in the aftermath of expensive negative trials[57,74,76,83], would result in abandoning this challenging, but utterly important, research topic before it is fully and adequately investigated. Risk is a continuous, not a dichotomous, concept; periodontitis is an inflammatory stressor with potential impact on several body systems; and a plethora of interesting questions remain unanswered.

## Acknowledgements

Research support to Dr Papapanou was provided by the National Institutes of Health/National Institute for Dental and Craniofacial Research, Bethesda, Maryland, USA (grants DE014338, DE015649, DE021820, DE022568, DE024735) and Colgate-Palmolive, Piscataway, NJ, USA.

## Conflict of Interests

None.

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