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Diabetes, periodontitis, and the subgingival microbiota

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Both type 1 and type 2 diabetes have been associated with increased severity of periodontal disease for many years. More recently, the impact of periodontal disease on glycaemic control has been investigated. The role of the oral microbiota in this two-way relationship is at this stage unknown. Further studies, of a longitudinal nature and investigating a wider array of bacterial species, are required in order to conclusively determine if there is a difference in the oral microbiota of diabetics and non-diabetics and whether this difference accounts, on the one hand, for the increased severity of periodontal disease and on the other for the poorer glycaemic control seen in diabetics.

Keywords: *diabetes*; *periodontitis*; *subgingival microbiota*; *metabolic control*

Diabetes and periodontitis are both complex chronic diseases for which there is substantial evidence for a bidirectional relationship. There is clear evidence that diabetics have an increased prevalence and severity of periodontitis. There is also evidence to suggest that individuals with periodontitis have an increased prevalence of diabetes, and that diabetics with periodontitis have poorer glycaemic control. The prevalence of diabetes is growing rapidly worldwide, especially in developing nations that are undergoing rapid urbanisation. It has been estimated that in the year 2000, 171 million people worldwide suffered from diabetes and that this will increase to 366 million by 2030 (1).

Classic diabetic complications include microangiopathy, retinopathy, nephropathy, neuropathy, and accelerated atherosclerosis. In combination with the systemic complications, there are often oral manifestations and complications that include xerostomia, mucosal diseases such as recurrent aphthous ulceration, as well as burning mouth syndrome (2). Xerostomia is likely to result from depletion of extracellular fluids as a result of polyuria and may predispose to further oral complications such as dental caries, mucosal infections, and difficulty masticating. Periodontitis has been described as the 'sixth complication of diabetes' (3). These complications result from metabolic derangements, especially hyperglycaemia.

An increase in the prevalence and severity of periodontitis has been observed in diabetics (4, 5) and has been confirmed in a recent meta-analysis of 23 studies (5). In type 1 diabetics, an increase in the severity of periodontal diseases has been shown across most age ranges. The strength of this association appears to vary with age. Age itself has been shown to be a risk factor for periodontitis (6), and is likely to be a confounder in studies investigating the link. A study of type 1 diabetics aged 19–25 years showed no differences between diabetics and non-diabetics in terms of oral hygiene status; however, the diabetic group did show higher frequencies of inflamed buccal/lingual gingiva and gingival recession, which suggests an altered inflammatory response to plaque (7). In a larger study, approximately 10% of type 1 diabetics aged between 13 and 18 years had periodontitis, compared with only 1.7% of non-diabetics (8). More extensive and severe periodontitis was observed in 40–49 year olds with long-standing insulin-dependent diabetes (25.6 ± 9.8 years) than in non-diabetic controls (9). However, no statistically significant differences were noted between diabetics and non-diabetics aged 50–59 or 60–69 years. In fact, alveolar bone loss was not significantly different between diabetics aged 40–49 years and 60–69 years. It appears that the age of onset of diabetes and duration of disease may be factors as the older age group in this study had a shorter average disease duration (18.6 ± 11.2 years).

Type 2 diabetes has also been shown to be a risk factor for periodontal diseases. This relationship is most clearly demonstrated in the Pima Indian population of Arizona. This population has the world's highest incidence and

prevalence of type 2 diabetes. A study of the association between diabetic status and periodontal conditions in 1,342 individuals showed an increased risk for period-ontitis with an odds ratio of 3.43 (95% CI 2.28–5.16) for alveolar bone loss, after adjusting for demographic variables and several oral health indices including the plaque index (10). It was further demonstrated in a 2-year longitudinal study that, in the Pima Indian population, type 2 diabetics had increased progression of alveolar bone loss (11).

The degree of metabolic control by diabetic patients is likely to influence susceptibility to periodontitis, as it is hyperglycaemia that leads to the characteristic complica-tions of diabetes. Tervonen and Oliver (12) demonstrated this in a cross-sectional study into the association between long-term diabetic control and periodontal status. Diabetics were assessed using HbA_{1c} and for plaque, calculus, probing depth, and attachment loss. Based on their control of blood glucose levels, they were grouped into 'good control', 'moderate control', and 'poor control' of blood glucose levels. It was found that the prevalence of severe attachment loss increased with decreasing control of diabetes. This study found that 10% of well controlled and 27% of poorly controlled diabetics had loss of attachment greater than 5 mm (12). These findings have been confirmed by other studies – both cross-sectional (13) and longitudinal (14, 15).

A meta-analysis of 18 cross-sectional, three prospective cohort, and two clinical trials confirms the overall conclusion that diabetics have significantly higher sever-ity and prevalence of periodontitis (5). The gingival index and bleeding index were not significantly different among diabetics versus non-diabetics. However, the overall difference in average probing depths and attachment loss was significantly greater in diabetics.

The role of plaque in periodontal disease

The causal role of dental plaque in gingivitis was clearly demonstrated in the landmark experimental gingivitis study of Loe et al. (16). For obvious ethical reasons, such a clear cause and effect relationship has not, as yet, been established for chronic periodontitis in humans. Never-theless, an overwhelming weight of circumstantial evi-dence exists showing that if there is no plaque, there is no disease (17–19). This strongly indicates a causal role for the bacteria within the plaque. Over the past two decades, however, it has become apparent that it is the nature of the host response to plaque that determines loss of periodontal attachment and alveolar bone (20). In this context it can be seen that periodontal disease progres-sion results from both bacterial virulence factors as well as host inflammatory mechanisms.

Despite widespread acceptance of the specific plaque hypothesis (21) in the etiology of chronic periodon-titis, periodontal pathogens are frequently detected in periodontally healthy individuals (22). Nevertheless, once formed, deep periodontal pockets could provide a suitable environment that further selects for specific anaerobic bacterial complexes (23). Factors that alter this subgingival environment, including inflammation and the myriad of cytokines and mediators produced, could therefore influence the composition of the sub-gingival biofilm. In this context, factors such as diabetes that alter the nature of the immune/inflammatory re-sponse could conceivably influence which bacterial com-plexes form subgingivally. At this stage, however, the effect of diabetes on the composition of the subgingival plaque is unclear. While certain bacterial species are more commonly found in diabetic patients, it is more difficult to determine whether this occurs because of direct alterations to the subgingival microenvironment or whether it occurs indirectly by alterations to the host response. Diabetic individuals may be more susceptible to chronic periodontitis as a result of hyperglycaemia altering the subgingival microenvironment such that bacterial species that are more pathogenic in nature will become dominant. Alternatively, diabetes may alter the host response to plaque resulting in more tissue destruction.

Effect of diabetes on the subgingival microbiota

Several studies have investigated the composition of plaque in diabetics compared with non-diabetics. In-creased numbers of so-called periodontal pathogens have been isolated from periodontal pockets of diabetic patients (24, 25), although the specific differences in the microbiota of diabetics compared with non-diabetics is not clear. Thorstensson et al. observed significantly greater numbers of *Porphyromonas gingivalis* in diabetics compared to controls, although no differences were seen with *Actinobacillus* (*Aggregatibacter*) *actinomycetemco-mitans*, *Campylobacter rectus*, *Capnocytophaga* spp., *Eikenella corrodens*, *Fusobacterium nucleatum*, and *Pre-votella intermedia* (26). Other studies, including long-itudinal studies found no such association (27–30). More recently, the periodontal microbiota of diabetics and non-diabetics was compared using checkerboard DNA–DNA hybridisation. Of the 17 species tested for, *Treponema denticola*, *Streptococcus sanguinis*, *Prevotella nigrescens*, *Staphylococcus intermedius*, and *Streptococcus oralis* levels were elevated in the supragingival plaque of diabetics compared with non-diabetics, although no significant differences were found in subgingival plaque samples (31). Subgingival infection patterns were also found to be similar in type 1 diabetics and non-diabetic controls of comparable periodontal status (32). In contrast, using similar methodology, Ebersole et al. showed significantly increased frequency of *P. gingivalis*, *Campylobacter* spp., and *A. actinomycetemcomitans* in the subgingival plaque of diabetics compared with

non-diabetics (33). A higher prevalence of *P. gingivalis* was also demonstrated in type 2 diabetics compared with non-diabetic controls using polymerase chain reaction (PCR) (34). Further, *P. gingivalis* with the Type II *fim A* gene, a more virulent clone, was associated with more extensive periodontitis in type 2 diabetics (35).

The subgingival microenvironment is determined, in part, by the composition of gingival crevicular fluid (GCF) and, in part, by the composition of the subgingival microbiota itself. The GCF is a serum product, which also contains all of the components of an inflammatory exudate including complement, immunoglobulins, inflammatory mediators, and immune cells. The flow of GCF rapidly increases with the onset of inflammation (36). This has the potential to alter the subgingival microenvironment and the nature of the microbiota that reside within it (37). During the pathogenesis of gingivitis and periodontitis, the ecology of the subgingival microenvironment changes from one in which there is a shallow sulcus and minimal flow of GCF where Gram-positive facultative anaerobic cocci and rods predominate, to one in which there is a deepened pocket with an increased supply of nutrients from GCF where predominantly anaerobic and pathogenic species predominate in the biofilm. While a similar shift in ecology would be expected in diabetic periodontal patients, there may indeed be differences in the subgingival microenvironment in diabetic patients compared with non-diabetics.

The glucose content of GCF in diabetics has been shown to be elevated compared with non-diabetics (38). This could provide an altered source of nutrition for subgingival microorganisms and subsequently modify the proportions of certain species within the biofilm. Furthermore, the immune response to periodontal pathogens may be altered or impeded in diabetics, which could lead to the overgrowth of certain species. Advances in the understanding of biofilms indicate that there is a highly complex interplay between many different species with certain, more virulent, organisms tending to coaggregate (39, 40). At this stage however, further studies of a longitudinal nature and investigating a wider array of bacterial species are required in order to conclusively determine if there is a difference in the biofilm of diabetics and non-diabetics.

Effect of diabetes on the host response

Collagen is the most abundant protein in the animal kingdom and provides structure to all periodontal tissue components including gingiva, periodontal ligament, cementum and alveolar bone, as well as blood vessels. Connective tissue metabolism in diabetics is altered in comparison to non-diabetics. Diabetes and hyperglycaemia may, through its effect on collagen metabolism, tissue homeostasis, and wound healing, play a role in greater loss of attachment. Hyperglycaemia has the potential to cause alterations to the structure of collagen and disrupt its synthesis, modifying the course or nature of periodontal diseases. Willershausen-Zonnchen et al. demonstrated a dose-dependent reduction in collagen and glycosaminoglycan synthesis, the two most common components of the extracellular matrix, in cultured human gingival fibroblasts as a result of elevated glucose concentrations (41). Alterations to these components reduce the capacity of connective tissue to remodel and so can affect the progression of periodontal diseases.

Hyperglycaemia in diabetes is known to induce changes in collagen via advanced glycation end products (AGEs) (42). Collagen molecules become cross-linked via stable bonds, which cause the collagen to become less soluble, less susceptible to proteolytic enzymes, and more rigid (43). While this may not appear to be to the detriment of the attachment apparatus, these effects are noted in the vasculature of the periodontium with signs of microangiopathy that are characteristic of diabetes. Several studies have documented significant increases in the thickness of the basement membrane of gingival capillaries (44, 45). Frantzis et al. in an electron microscopy study noted that capillary basement membranes of diabetic periodontal patients were approximately four times thicker than those of non-diabetic periodontal and non-periodontal patients (44). Listgarten et al. made similar observations (45).

On the other hand, altered tissue homeostasis in diabetics may occur via a potential increase in collagen degradation. Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases with the primary purpose of degradation of the extracellular matrix (46). Collagenases are a subclass of matrix metalloproteinases that cleave collagen molecules, enabling them to become denatured, further degraded, and phagocytosed. Collagenases are known to be increased in the periodontium of diabetics (47). These increases in collagenases may be reversed with good glycaemic control (48).

The alterations in connective tissue metabolism that have been observed in diabetics, with an increase in collagenase production, along with well established microangiopathic effects are likely to be important factors in the pathogenesis of periodontitis as a diabetic complication. These alterations are likely to not only reduce the health of these tissues but also inhibit wound healing such that the periodontal tissues are more susceptible to periodontal pathogens, resulting in greater severity, extent, and progression of periodontal diseases.

The role of the polymorphonuclear neutrophil in the defence against plaque bacteria is well established. In fact, the neutrophil has been described as the first-line of defence against periodontal pathogens (49). Defects in neutrophil function and recruitment in diabetics have

been observed (50–52). Bissada et al. showed that peripheral blood neutrophils have reduced chemotactic activity in type 1 diabetics with severe periodontitis when compared with diabetics with moderate periodontitis and non-diabetics with severe periodontitis (50). They also showed that the phagocytic activity of peripheral blood neutrophils of type 1 diabetics with localised periodontitis was lower than non-diabetics with localised periodontitis.

Monocytes/macrophages are also altered in diabetics, potentially exacerbating the progression and severity of periodontal disease. Diabetics may possess a hyper-responsive monocyte/macrophage phenotype in which there is increased synthesis and secretion of proinflammatory mediators such as TNF-α, IL-1β, and PGE$_2$. Salvi et al. demonstrated this by culturing peripheral blood monocytes from type 1 diabetics and healthy controls with varying degrees of periodontitis and examining the production of TNF-α in response to LPS (53). They demonstrated that monocytes from diabetics have between 24 and 32 times more TNF-α production (depending on the concentration of LPS) than non-diabetic controls, irrespective of periodontal status. The same research group also showed significantly higher levels of IL-1β and PGE$_2$ from cultured monocytes and in the GCF of type 1 diabetics compared with non-diabetics who were matched for periodontal disease severity (54). This exaggerated inflammatory response would be likely to stimulate increased secretion of matrix metalloproteinases, with subsequent increased degradation of the extracellular matrix of the periodontal tissues, leading to increased loss of attachment (55), although a recent study failed to show this experimentally (56).

Altered tissue homeostasis, wound healing, and host inflammatory response are likely to be the result of accumulation of AGEs within the gingival/periodontal tissues. The interaction of AGEs with the receptor for AGE (RAGE) may, in part, add further explanation (57). RAGE is a member of the immunoglobulin superfamily of cell surface molecules. Under normal healthy conditions, RAGE is present in endothelial cells, smooth muscle cells, neurons, and monocytes. However, in diabetes, expression of RAGE is markedly increased (57). AGEs can then bind to RAGE, leading to further complications such as the development of vascular lesions, increased vascular permeability, increased expression of adhesion molecules, and increased migration and activation of monocytes (57).

Periodontitis and diabetic control

As outlined above there is clear evidence that diabetics have increased prevalence and severity of periodontitis and that individuals with periodontitis have an increased prevalence of diabetes. Indeed, unstable periodontitis may have the potential to worsen glycaemic control in diabetics. Taylor et al. showed that in the Pima Indian population of Arizona individuals with severe periodontitis had up to 13 times greater risk of worsening glycaemic control after 2 years, depending on age (58).

Interventional studies, in which glycaemic control was assessed in participants with pre-existing periodontitis and diabetes before and after a course of periodontal therapy, provide insight into this relationship. Randomised controlled trials have demonstrated significant improvements to glycaemic control in type 1 (59) and type 2 diabetics (60, 61) following non-surgical periodontal therapy. Several other studies, however, failed to support this including randomised controlled trials investigating type 1 diabetics (62, 63) and type 2 diabetics (64).

It has been suggested that antibiotics, when used as part of periodontal therapy in diabetics, may have additional benefits in terms of periodontal and glycaemic control outcomes. Grossi et al. found that only non-surgical periodontal therapy with the addition of systemic doxycycline (100 mg for 2 weeks) had a significant effect on glycaemic control, while non-surgical periodontal therapy without systemic antibiotics did not (65). However, this finding is questioned in a study by Rodrigues et al., which found that patients who received non-surgical periodontal therapy alone had significantly reduced HBA$_{1c}$ after 3 months, while patients who received non-surgical periodontal therapy with the addition of amoxicillin/clavulanic acid did not (61).

The significant heterogeneity of the above-mentioned studies in terms of design and findings means there is difficulty in drawing clear conclusions. Janket et al. conducted a meta-analysis of interventional studies that evaluated the effect of periodontal treatment on glycaemic control. They included 10 studies involving type 1 and type 2 diabetics and found that the reductions in HbA$_{1c}$ were small ($<1\%$) and not statistically significant. In type 2 diabetics there was a decrease in HbA$_{1c}$ of 0.4% following non-surgical periodontal therapy alone and a decrease of 0.7% with the addition of antimicrobials (66). More recently, two further meta-analyses were conducted (67, 68). The analysis by Teeuw et al. (68) included five studies with a total of 371 type 2 diabetic patients and Simpson et al. (67) included 244 participants, predominantly type 2 diabetics, from three studies. Both meta-analyses showed a statistically significant improvement of 0.4% in HbA$_{1c}$ level. Simpson et al. (67) found no added benefit from the use of adjunctive antibiotics in lowering

HbA$_{1c}$ or improving clinical parameters. Put in context, every percentage point reduction in HbA$_{1c}$ correlates with a 35% reduction in microvascular complications (69). Therefore, it appears likely that periodontal therapy has a clinically measurable benefit to the long-term glycaemic control of diabetics.

The biological basis for periodontal disease influencing glycaemic control in diabetics is feasible. Inflammatory cytokines such as IL-1, IL-6, and TNF-α, which are all important mediators of periodontal inflammation, also play a role in glucose and lipid metabolism. Plasma concentrations of IL-6 and TNF-α may be increased in obese individuals and in type 2 diabetics. Notably, IL-6 and TNF-α act as adipokines that serve to promote catabolism and weight loss and are also involved in insulin resistance. It has been proposed that the inflamed periodontium may act as an endocrine-like source of inflammatory mediators such as TNF-α, IL-1, and IL-6, which can subsequently increase insulin resistance (70).

Patients with chronic periodontitis may have a substantial surface area of inflamed periodontal tissue. Using a measure of periodontal inflamed surface area (PISA), Nesse et al. showed a dose-response relationship between PISA and HbA$_{1c}$ levels, suggesting a link (71). Numerous studies have demonstrated that individuals with chronic periodontitis have an elevated state of systemic inflammation. C-reactive protein (CRP), which is a widely used marker of systemic inflammation, has been shown to be elevated in patients with chronic periodontitis even when controlling for confounding factors (72–75). Similarly, other inflammatory mediators including TNF-α and IL-6 have also been shown to be elevated in the blood of individuals with chronic periodontitis (76–78).

Transient bacteraemias occur in all individuals and it is suspected that individuals with periodontitis may experience a higher frequency of bacteraemia due to the large surface area of ulcerated pocket epithelium that is in constant contact with the plaque biofilm (79). Bacteraemias occur both in patients with gingivitis and periodontitis following routine oral hygiene procedures such as flossing and may even occur subsequent to mastication (80, 81). A link between bacteraemia of periodontal origin and systemic complications such as atherosclerosis and adverse pregnancy outcomes has been postulated for over 20 years (82, 83). While the systemic inflammation hypothesis may apply to these complications, other mechanisms such as direct infection (84) and molecular mimicry (85) have been postulated. There is now robust evidence supporting molecular mimicry as a biological mechanism linking chronic periodontitis to accelerated development of atherosclerotic plaques (86, 87). The possibility of a direct effect of bacteraemia and diabetic control has not been explored.

Conclusion

While the epidemiological association between periodontitis and diabetes is becoming relatively clear, the biological mechanisms of the association are yet to be fully elucidated. As a clearer understanding of the influence of periodontal inflammation on the composition of the subgingival biofilm emerges, the link between periodontitis and diabetes will also become better understood. It is recognised that as progression of periodontal disease occurs, there is a simultaneous increase in numbers of anaerobic species in subgingival plaque, as well as an increase in inflammation, both local and systemic. Whether it is because of the effect of diabetes on subgingival plaque or the effect on the host response that results in greater disease progression remains uncertain. Indeed, both mechanisms are probably involved. Likewise, the mechanism behind the effect of periodontitis on diabetic control is equally unclear. The importance of systemic inflammation exacerbated by periodontal inflammation is compelling. The role of bacteria in these processes has not been fully explored and remains the subject of future research.

Conflict of interest and funding

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