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Periodontitis and Type 2 Diabetes: Is Oxidative Stress the Mechanistic Link?

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Abstract

Periodontitis is a common, chronic inflammatory disease initiated by bacteria which has an increased prevalence and severity in patients with type 2 diabetes. Recent studies indicate that the co-morbid presence of periodontitis can, in turn, adversely affect diabetic status and the treatment of periodontitis can lead to improved metabolic control in diabetes patients. Current evidence points to a *bidirectional interrelationship* between diabetes and inflammatory periodontitis. The importance of oxidative stress-inflammatory pathways in the pathogenesis of type 2 diabetes and periodontitis has recently received attention. Given the bidirectional relationship between these two conditions, this review discusses the potential synergistic interactions along the oxidative stress-inflammation axis common to both type 2 diabetes and periodontitis, and the implications of this relationship for diabetic patients.

Keywords

Diabetes, periodontitis, oxidative stress, inflammation.

Introduction

Periodontitis is a common, chronic inflammatory disease, initiated by plaque bacteria which affects the tissues that support the teeth. Type 2 diabetic patients have an increased prevalence^{1,2,3,4,5,6,7,8} and severity^{7,9,10} of periodontitis.

Recent studies indicate that the co-morbid presence of periodontitis can, in turn, adversely affect diabetic status.^{10,11} Treatment of periodontitis is reported to improve metabolic control in diabetes, as measured by HbA1c levels.^{12,13,14,15} Evidence points to a bidirectional interrelationship between diabetes and inflammatory periodontitis.¹⁶ This review examines the evidence that oxidative stress is a key pathological link between periodontitis and type 2 diabetes and that disease outcomes are exacerbated when both diseases co-exist.

Oxidative stress

Free radicals are "species capable of independent existence that contain one or more unpaired electrons".¹⁷

The unpaired electrons of free radicals confer an inherent instability and high reactivity potential with other biomolecules. Reactive Oxygen Species (ROS) is a term collectively describing oxygen radicals and other non-radical but reactive oxygen derivatives, many of which are found in living organisms (Table I).¹⁸

Table I: Reactive Oxygen Species (ROS) in Living Organisms (adapted from reference 18).

| Radicals | Non-Radicals |
|--------------|--|
| Hydroxyl | Peroxynitrite |
| Superoxide | Hypochlorous acid |
| Nitric Oxide | Hydrogen Peroxide |
| Thyl | Singlet Oxygen |
| Peroxyl | Ozone |
| Lipid peroxy | Lipid hydroperoxide |
| | ONOO ⁻ |
| | HOCl |
| | H ₂ O ₂ |
| | ¹ Δ _g (⁻¹ O ₂) |
| | O ₃ |
| | LOOH |

ROS are continuously generated in the body during mitochondrial oxidative metabolism due to electron leakage from their carriers within the mitochondrial electron transport chains. In addition, ROS are generated by the NADPH-oxidase enzyme complex on the inner lipid membrane of inflammatory cells and other cell types. In health, it is now known that ROS, in addition to their bactericidal function, play an important role in normal homeostasis by controlling gene expression, cellular signal transduction and maintaining vascular health.¹⁹

The delicate redox balance within cells and tissues essential for physiological and biochemical homeostasis is maintained by antioxidant mechanisms including a series of enzymes that can degrade ROS and dietary-derived, small molecule antioxidants including vitamins A, C and E.²⁰ When antioxidant defence systems are compromised or ROS-production is excessive, a state of "oxidative stress" arises and this state is an important contributing factor to tissue damage in many chronic human diseases (atherosclerosis, cancer, neurodegenerative disorders, and ageing),²⁰ including periodontitis and diabetes.^{17,21,22,23}

Cell and tissue damage can result directly through reactivity of ROS with biomolecules leading to alterations in the structure and function of DNA, lipids and proteins. In addition, more subtle increases in ROS concentration within cells allows the activation of REDOX sensitive transcription factors such as activating protein-1 (AP-1) and nuclear factor kappa B (NFκB).²⁴

The NFκB family of redox-sensitive transcription factors play a critical role in inflammatory, apoptotic and immune responses²⁵ whereas AP-1 regulates the activity of a wide variety of pro-inflammatory genes including IL-2, IL-8, TNFα, matrix metalloproteinases and adhesion molecules.

The investigation and establishment of the role played by oxidative stress in disease pathogenesis requires the determination of oxidative status. Direct measurement of free radicals is problematic due to their short half-lives and high reactivity, therefore oxidation products of biomolecules are employed as indirect markers of oxidative stress (Table II).²⁶

Table II: Some Commonly Used Markers of Oxidative Damage.

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|--|
| <p>Lipid peroxidation</p> <ul style="list-style-type: none"> • Lipid peroxides • Aldehydes formed from oxidation of polyunsaturated fats (e.g. Malondialdehyde) • Acrolein • Isoprostanes (eg. F2-isoprostanes from arachadonic acid) |
| <p>DNA oxidation</p> <ul style="list-style-type: none"> • 8-hydroxy-2-deoxyguanosine (8-OHdG) • DNA strand breaks (Comet assay) |
| <p>Protein oxidation</p> <ul style="list-style-type: none"> • Protein carbonyls |

Determination of antioxidant status is more amenable to study and assays have been developed which measure the total antioxidant capacity (TAOC) of biological fluids and tissues by (a) measuring the susceptibility of biological fluids to oxidation by the addition of pro-oxidants and monitoring by an exogenous, oxidisable substrate²⁷ or (b) assessing the ability of a biological sample to quench a pre-formed radical over a measured inhibition time.²⁸

Periodontitis

Periodontitis encompasses chronic inflammation of the supporting tissues (gingival, periodontal ligament, cementum and bone) of the teeth, initiated by inadequate oral hygiene with accumulation and maturation of a sub-gingival plaque biofilm containing gram-negative, anaerobic bacteria.²⁹ The prevalence of severe periodontitis is 5-20% of populations that have been examined worldwide³⁰ and therapy involves physical disruption of the plaque biofilm with oral hygiene education to prevent plaque re-accumulation. However, an exaggerated inflammatory and immune response to the presence of specific pathogenic bacteria (eg. Porphyromonas gingivalis) is the key determinant of individual susceptibility to periodontitis.³¹ This "hyper-inflammatory" state results in elevated levels of local neutrophil-derived, degradative enzymes, circulating cytokines and C-reactive protein (CRP).³² Enzyme and cytokine-mediated destruction of the bone and collagen-rich connective tissue support of the teeth results and ultimately leads to tooth loss in severe cases.³³

Oxidative Stress in Periodontitis

Neutrophils are the most prominent cells of the gingival inflammatory infiltrate in patients with periodontitis.³⁴ Binding of bacteria directly or indirectly by the surface receptors (Toll-like receptors [TLR] and Fcγ-receptors [Fcγ-R]) of neutrophils triggers phagocytosis and superoxide radical formation which may subsequently be converted to hydrogen peroxide and the highly reactive hydroxyl radical. The generation of oxygen free radicals activates bactericidal enzymes within the phagosome³⁵ and within this protective environment, bacteria are destroyed. However, extracellular release of ROS by neutrophils is recognised as an important factor contributing to the tissue damage in periodontitis.^{36,37} Peripheral blood neutrophils from periodontitis patients exhibit spontaneous ROS release in addition to hyper-reactivity (post-TLR or Fcγ-R stimulation) compared with healthy controls.^{38,39,40} While the stimulated hyper-reactivity may be partially reduced by periodontal therapy, the spontaneous production of extracellular ROS is not.³⁸ Recently, neutrophils from periodontitis patients have also been shown to exhibit a distinct molecular phenotype,⁴¹ which together with the data from the functional studies, suggests that periodontitis is associated with peripheral activation of neutrophils.

Further evidence for a pathogenic role and peripheral impact of ROS comes from studies which show that periodontitis is negatively associated with serum antioxidant concentrations.⁴² Both cross-sectional case control and longitudinal experimental studies have also shown a reduction in TAOC both within the local periodontal tissues and in plasma compared to controls,^{43,44} together with increased levels of an antioxidant enzyme (SOD) within the gingivae (45). Improvement in both local and peripheral TAOC status following successful treatment for periodontitis^{43,44} support the epidemiological data⁴² and reinforces the case for systemic effects of ROS in periodontitis.

Locally within the periodontal tissues, the excessive release of ROS and alteration in redox balance can result in local tissue damage directly (eg. oxidation of extracellular and cellular macromolecules) and indirectly via activation of redox-sensitive nuclear transcription such as NFκB and AP-1 leading to an amplification of inflammatory and immune processes. There is now accumulating data for the presence of periodontitis-associated oxidative damage within periodontal tissues in both human^{46,47} and animal studies.⁴⁸ However, the evidence for systemic alterations in redox status in periodontitis suggests that these patients either have a lower threshold antioxidant defence capacity and/or are predisposed towards exaggerated ROS-release at peripheral sites.⁴³ Investigation of peripheral oxidative damage in periodontitis is in its infancy but recent reports have suggested increased levels of protein carbonyls in peripheral blood and saliva, and oxidative damage within tissues such as liver.^{49,50,51}

Diabetes Mellitus

Diabetes mellitus encompasses a group of metabolic disorders characterised by hyperglycaemia secondary to defects in insulin secretion, insulin action or both (Table III).⁵² Type 2 diabetes has reached epidemic proportions with 1.8 million affected people in the UK (3% of the population) and up to a further million with undiagnosed disease (Diabetes in the UK 2004, A report from Diabetes UK, October 2004). Furthermore, patients with type 2 diabetes are at increased risk for macrovascular and microvascular damage including cardiovascular disease, retinopathy and nephropathy.

Table III: Classification of Diabetes Mellitus by Aetiology – (American Diabetic Association, 2004).

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|--|
| I. Type 1 diabetes (β -cell destruction, usually leading to absolute insulin deficiency) |
| A. Immune mediated |
| B. Idiopathic |
| II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance) |
| III. Other specific types-including |
| Genetic defects of insulin action/cell function |
| Diseases of the exocrine pancreas |
| Endocrinopathies |
| Drug- or chemical-induced |

The aetiology of type 2 diabetes appears to be multifactorial. A genetic component contributes to individual susceptibility for the development of type 2 diabetes.⁵³ Metabolic syndrome, the characteristics of which are impaired glucose tolerance, obesity, hypertension and dyslipidaemia, is associated with an increased risk for the development of diabetes by a factor of 2.99 and may be considered a 'pre-diabetic' state.⁵⁴ Type 2 diabetes and the 'pre-diabetic' metabolic syndrome are associated with obesity, physical inactivity and a high glucose, high fat, low fibre diet.

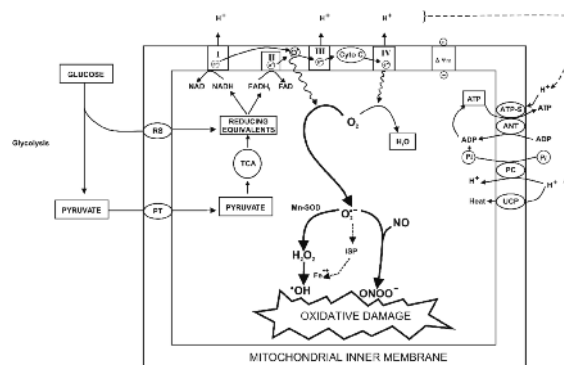
Insulin resistance is the initial abnormality in glycaemic control found in type 2 diabetes. It may be present for a number of years before the onset of diabetes and is a feature of metabolic syndrome.⁵⁵ However, over time chronic hyperglycaemia and 'glucose toxicity' results in hypofunction of the pancreatic B-cells and an exacerbation of glucose intolerance. Insulin resistance is associated with stimulation of innate immunity and an upregulated inflammatory state⁵⁶ leading to increased levels of the cytokine TNF- α which induces serine-phosphorylation of the insulin receptor and contributes to defective insulin signalling.⁵⁷

Diabetes and Oxidative Stress

Current evidence suggests that oxidative stress may be the underlying pathological condition associated with the development of pre-diabetic and diabetic conditions, and may also be responsible for the complications of diabetes.⁵⁸ It is considered that the stimulated inflammation observed in the pre-diabetic and diabetic state is a direct manifestation and consequence of chronic pre-existing oxidative stress.⁵⁹ Indirect markers of oxidative stress including markers of lipid peroxidation⁶⁰ and DNA damage⁶¹ are significantly higher in diabetic patients than healthy controls. Diabetic patients have lowered antioxidant defences,⁶² total antioxidant status of plasma⁶³ and total antioxidant levels are inversely related to the development of diabetic complications.⁶⁴

Dietary factors, especially glucose and fat intake induce oxidative stress, triggering redox-sensitive cell signalling and upregulation of inflammation.⁶⁵ The consequences of repeated intake of high glucose, high fat macronutrients exceeding the energy requirements of the body is associated with ROS production at the mitochondrial level, with hyperglycaemic states leading to excess ROS generation (Figure 1).^{66,67} Interestingly, high glucose levels are also associated with enhanced NADPH oxidase activity and ROS production in neutrophils⁶⁸ and endothelial cells.⁶⁹ High glucose levels also contribute to the production of advanced glycation end-products (AGEs), created by the non-enzymatic glycation and oxidation of proteins, which, upon binding to their surface receptor RAGE, results in further ROS production by various cell types.⁷⁰

Figure 1. A model of mitochondrial ROS production in hyperglycaemia (adapted from Brownlee, 2004 & Green *et al.*, 2004 (66, 67)). Normal concentrations of glucose cause the production of electron donors (reducing equivalents) such as NADH and FADH₂ within mitochondria. These may enter mitochondria via redox shuttles (RS) or be derived from the TCA cycle subsequent to uptake of pyruvate by the pyruvate transporter (PT). Electrons are passed through the respiratory chain (complexes I-IV) to oxygen, finally producing water. This process leads to protons being pumped across the inner mitochondrial membrane and the production of an electrochemical potential gradient (membrane potential; $\Delta\Psi_m$). This gradient is reduced by ATP synthase (ATP-S) which produces ATP and allows the re-entry of protons into the mitochondrial matrix. ATP and ADP are exchanged across the inner membrane via adenine nucleotide transporter (ANT) and inorganic phosphate (Pi) enters via the phosphate carrier (PC). Protons may also recycle without ATP formation via uncoupling proteins (UCP). **High glucose concentrations** will lead to the presence of higher levels of electron donors, excessive electrons entering the respiratory chain and subsequent increase in the membrane potential until a critical threshold is reached. At this point, electron transfer at complex III is inhibited causing electrons to backup to ubiquinone (Q). Single electrons are then diverted to oxygen, causing the generation of superoxide which may then lead to the production of hydroxyl radicals (via H₂O₂ produced by Type 2 superoxide dismutase [Mn-SOD] and presence of Fe²⁺ liberated from iron sulphur proteins [ISP] by superoxide), peroxynitrite (by reaction of superoxide with nitrous oxide) and oxidative damage. (Cyto C, cytochrome C).



The excessive levels of ROS produced in diabetes are the proximal step in the activation of stress-sensitive signalling pathways (eg. NF κ B) and with other cell-signalling pathways (hexosamine and PKC) which are also associated with up-regulation of pro-inflammatory cytokines,⁶⁶ diabetic complications (eg. vascular disease, retinopathy and diabetic nephropathy)^{71,72,73} and insulin resistance.⁷⁴ More directly, excess mitochondrial superoxide production in pancreatic B-cells results in decreased insulin secretion.⁷⁵

Thus, evidence suggests that insulin resistance may develop as a result of oxidative stress and redox-stimulated upregulation of inflammation generated by the repeated consumption of high glucose, high fat foods, exacerbated by a lack of physical activity and associated obesity. Chronic hyperglycaemia and dyslipidaemia result in saturation of cellular anti-oxidant capacity with ongoing stimulation of redox-sensitive cell signalling pathways and down-stream activation of biochemical pathways associated with the development of diabetes and diabetic complications.

Inter-Relationship between Periodontitis and Type 2 Diabetes

Periodontitis has been identified as the sixth complication of diabetes⁷⁶ and its prevalence in type 2 diabetic patients is more than twice that of non-diabetic patients.^{1,2,3,4,5,6,7,8} Diabetic patients display an increased severity of disease^{7,9,10} with severity being related to diabetic control⁷ but unrelated to diabetic duration.⁹ However, periodontitis appears to have a reciprocating negative impact on diabetic status^{10,11} and significant relationships between periodontitis and both impaired glucose tolerance⁷⁷ and diabetic retinopathy have been reported.⁷⁸ Furthermore, periodontitis patients have been reported to have higher resting plasma glucose levels than control patients⁷⁹ and experimental periodontitis increases blood glucose levels in diabetic rats.⁸⁰

That periodontitis is a strong independent predictor of mortality from ischaemic heart disease and the development of diabetic nephropathy has been suggested by a prospective, longitudinal study of 628 diabetic subjects (type 2) of the Pima Indian race.^{81,82} Support for this has come from several studies that have shown that improved periodontal health, achieved through periodontal therapy, improves the metabolic control of type 2 diabetes as measured by HbA1c levels.^{11,12,13,14,15}

The Oxidative Stress/Inflammation Axis Uniting Periodontal Disease and Diabetes

Thus, current evidence points to a *bidirectional interrelationship* between diabetes and periodontitis. The precise nature of this interrelationship is unclear. An upregulated inflammatory state has been proposed as the common mechanism underlying both conditions^{83,84} with an increase in cytokines, including TNF- α , postulated as a possible link.⁸⁵ We suggest that oxidative stress is a common factor in periodontal disease, type 2 diabetes and perhaps the 'pre-diabetic' condition and that the imbalance in redox control resulting independently from these disease states acts synergistically, and amplifies in a bi-directional manner the biochemical and clinical course of these diseases.

Excess ROS generated by peripherally primed neutrophils in the periodontitis state^{38,39} and reduced peripheral antioxidant levels^{42,43} may further tax an already compromised local and peripheral antioxidant defence in the prediabetic/diabetic state. When both conditions co-exist the balance is tipped towards stimulation of redox-sensitive pathways with downstream upregulation of inflammation and associated insulin resistance, compromising blood glucose control and contributing to the development of diabetic complications. On the other hand, the diabetic conditions of chronic hyperglycaemia and increased AGE formation, may impair antioxidant capacity^{62,63,64} and enhance NADPH oxidase activity and ROS production by neutrophils^{68,86} contributing to both direct and indirect oxidative damage to periodontal tissues in response to periodontal pathogens within the dental plaque biofilm.

AGE accumulation in the gingival tissues of diabetic animals associated with a state of enhanced oxidative stress within the tissues⁸⁷ and expression of the RAGE by gingival vascular endothelium and epithelium has been demonstrated at diseased sites from periodontitis patients with and without type 2 diabetes.⁸⁸ *In vitro* AGE formation on type I collagen significantly increased neutrophil adhesion and chemotaxis as well as having a priming effect on subsequent stimulation.⁸⁹ These results suggest that oxidation-dependent changes in vascular endothelium and collagen within the periodontal connective tissues could increase the numbers of PMNs entering, and retard their migration through the tissues, increasing their potential to produce tissue-damaging levels of ROS.

Interestingly, the formation of AGEs links diabetes to smoking, the most important risk factor for the development of periodontitis.⁹⁰ Nicotine, a metabolite found in high concentrations in the plasma of smokers, not only causes the development of advanced glycation end products^{91,92} but also induces an increase in the expression of RAGE by human gingival fibroblasts *in vitro*⁹³ thus providing a mechanistic link between diabetes, smoking and periodontal disease based upon oxidative stress.

Summary

The pathological conditions of periodontitis and prediabetes/diabetes are associated with the generation of oxidative stress. This oxidative stress causes direct damage at the biomolecular level and triggers destructive pro-inflammatory responses including the oxidative stress/inflammation axis. The co-existence of periodontitis and pre-diabetes/diabetes leads to a synergistic lowering of antioxidant capacity, augmenting in a bi-directional manner the pathological process underpinning both conditions.

Implications for Diabetic Patients with Periodontitis and Future Therapeutic Strategies.

The oxidative status of diabetic patients both systemically and locally within the periodontal tissues needs to be considered as part of treatment regimes. Efforts to develop therapeutic strategies aimed at limiting ROS production or increasing the rate of removal by antioxidant mechanisms in diabetic patients have been advocated.^{67,94}

Alpha-lipoic acid (ALA) has powerful antioxidant ability and ALA supplementation improves the antioxidant status of diabetics independent of glycaemic control.⁹⁵ ALA supplementation has been used successfully in Germany for decades to treat diabetic neuropathy.⁹⁶

The potential applications of local antioxidant therapy in the periodontal tissues have been illustrated by animal studies which demonstrate that application of the ALA homologue, N-acetyl cysteine, decreases the intensity of the neutrophil oxidative burst by a direct scavenging action.⁹⁷ Aminoguanidine, a nitric oxide synthase inhibitor, decreased the levels of inflammation within the periodontal tissues of animals with artificially-induced periodontitis.⁹⁸ Further work is required to develop treatment strategies aimed at improving the antioxidant capacity in the periodontitis state, especially in high-risk patients with diabetes.

Patients with diabetes need to be informed of their increased risk for periodontitis. Periodontal therapy should be a key consideration in the management of diabetic patients with co-morbid periodontitis, due to the potential negative impact of periodontitis on local and systemic oxidative status and glycaemic control. The importance of maintaining optimal glycaemic control in an effort to minimise metabolically generated ROS with their consequent deleterious effects on the periodontal tissues should also be emphasised to these patients.

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