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Abstract

Diabetes mellitus and periodontal disease are chronic diseases affecting a large number of populations worldwide. Changed bone metabolism is one of the important long-term complications associated with diabetes mellitus. Alveolar bone loss is one of the main outcomes of periodontitis, and diabetes is among the primary risk factors for periodontal disease. In this review, we summarise the adverse effects of diabetes on the periodontium in periodontitis subjects, focusing on alveolar bone loss. Bone remodelling begins with osteoclasts resorbing bone, followed by new bone formation by osteoblasts in the resorption lacunae. Therefore, we discuss the potential mechanism of diabetes-enhanced bone loss in relation to osteoblasts and osteoclasts.

Keywords

bone loss, diabetes mellitus, hyperglycemia, inflammation, osseous, osteoblast, osteoclast, periodontitis

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REVIEW

Diabetes mellitus related bone metabolism and periodontal disease

Ying-Ying Wu[1,2], E Xiao[2,3] and Dana T Graves[2]

Diabetes mellitus and periodontal disease are chronic diseases affecting a large number of populations worldwide. Changed bone metabolism is one of the important long-term complications associated with diabetes mellitus. Alveolar bone loss is one of the main outcomes of periodontitis, and diabetes is among the primary risk factors for periodontal disease. In this review, we summarise the adverse effects of diabetes on the periodontium in periodontitis subjects, focusing on alveolar bone loss. Bone remodelling begins with osteoclasts resorbing bone, followed by new bone formation by osteoblasts in the resorption lacunae. Therefore, we discuss the potential mechanism of diabetes-enhanced bone loss in relation to osteoblasts and osteoclasts.
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Keywords: bone loss; diabetes mellitus; hyperglycemia; inflammation; osseous; osteoblast; osteoclast; periodontitis

DIABETES: INTRODUCTION

Diabetes mellitus is a heterogeneous group of disorders and is characterized by high blood glucose levels.[1] Type 1 diabetes mellitus (T1DM) results from an absolute deficiency of insulin, which is most commonly due to auto-immunological destruction of the insulin-producing pancreatic β cells but which can be caused by other etiologies. In type 2 diabetes mellitus (T2DM), muscle, fat and other cells become resistant to the actions of insulin. This results in the activation of a compensatory mechanism that induces β cells to secrete more insulin. T2DM occurs when the compensatory increase in insulin is insufficient to maintain blood glucose levels within a normal physiological range.[2–3] By 2025, 300 million people are projected to be afflicted with diabetes worldwide, with a prevalence of 6.4%.[4–5] The countries with the most people suffering from diabetes by the year 2025 are predicted to be India, China and the United States. T1DM represents 5%–10% of the total number of diabetes cases worldwide[6] and is the main type of diabetes in youth, representing 85% or more of all diabetes cases in individuals younger than 20 years of age worldwide.[7] On average, males and females are equally affected with T1DM in young populations.[8] T2DM accounts for 90% of diabetes cases globally.[4] This disorder has traditionally been considered a metabolic disorder of adults; however, it has recently become more common in young adults, adolescents and occasionally, in children.[9]

PATHOGENIC MECHANISMS OF DIABETES

T1DM is a polygenic autoimmune disease that is characterized by the destruction of insulin-secreting pancreatic beta cells.[10] T1DM typically occurs as a consequence of a breakdown in immune regulation, resulting in an expansion of auto-reactive $CD4^+$ and $CD8^+$ T cells and auto-antibody-producing B lymphocytes and activation of the innate immune system, which collaborates to destroy insulin-producing beta cells.[11] In an animal model, $CD11c^+$ dendritic cells and ER-$MP23^+$ macrophages are the first cells to infiltrate the pancreas of non-obese diabetic mice, at approximately 3 weeks of age. At the same time, potentially pathogenic T cells can be detected surrounding the islet, resulting in peri-insulitis.[12] Genetic susceptibility and environmental triggers are thought to contribute to the development of T1DM.[13]

T2DM is a metabolic disorder that is characterized by hyperglycemia and altered lipid metabolism, which is caused by the inability of islet β cells to secrete adequate insulin in response to varying degrees of insulin resistance caused by over-nutrition, inactivity or obesity. Metabolic defects that contribute to the development of T2DM include an inability of islet β cells to compensate for high glucose levels that are associated with excess food intake, increased glucagon secretion and reduced incretin response, impaired expansion of subcutaneous adipose tissue, hypoadiponectinaemia, inflammation of adipose tissue, increased endogenous glucose production and the development of peripheral insulin resistance.[14] Chronic caloric excess is the primary pathogenic event that drives the development of type 2 diabetes in genetically and epigenetically susceptible individuals.[15–16]

PATHOGENIC CHANGES IN BOTH T1DM AND T2DM

Hyperglycemia

Hyperglycemia is due to impaired insulin secretion in T1DM and insulin resistance in T2DM. Beta cell destruction in T1DM and inadequate expression of glucose transporter 2 in T2DM are thought to contribute to hyperglycemia.[17] Several pathways are thought to exhibit increased activity under hyperglycemic conditions and to contribute to oxidative stress via the polyol pathway,[18] the hexosamine

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pathway [19–21] and activator of protein kinase C.[22] Hyperglycemia also leads to greater activation of the pro-inflammatory transcription factor, nuclear factor-kappa B (NF-κB), by protein kinase C *in vitro*.[19] Hyperglycemia also results in the oxidation of sorbitol by NAD^+, thereby increasing the cytosolic NADH:NAD^+ ratio and consequently inhibiting glyceraldehyde-3-phosphate dehydrogenase activation.

Advanced glycation end products

Advanced glycation end products (AGEs) are formed by the non-enzymatic reaction of glucose and other glycating compounds that are derived from glucose and increased fatty acid oxidation. Intracellular hyperglycemia is the primary initiating event in the formation of both intracellular and extracellular AGEs.[23] AGEs are derived from the intracellular auto-oxidation of glucose to glyoxal,[24] decomposition of the Amadori product (glucose-derived 1-amino-1-deoxyfrutose lysine adducts) and fragmentation of glyceraldehade-3-phosphate and dihydroxyacetone phosphate to methylglyoxal.[25] These reactive intracellular dicarbonyls (glyoxal, methylglyoxal and 3-deoxyglucosone) react with the amino groups of intracellular and extracellular proteins to form AGEs. Intracellular proteins that are modified by AGEs exhibit altered function. Extracellular matrix components that have been modified by AGE precursors interact abnormally with other matrix proteins and their receptors on cells. Several AGE receptors are linked to increased inflammation, including receptor for AGE (RAGE). Proteins can be structurally modified by glycosylation, thereby affecting their function. Alternatively, AGE binding to AGE receptors can induce the production of reactive oxygen species, the production of inflammatory cytokines such as tumour necrosis-alpha (TNF-α), and the activation of NF-κB.[26]

Lipotoxicity

Due to the presence of long-chain free fatty acids in the plasma, lipotoxicity is often increased in states of insulin resistance, thereby impairing β-cell secretory function [27–28] and contributing to β-cell apoptosis [29–30] and insulin resistance.[31] Muscle cells and hepatocytes are negatively affected by excessive amounts of fatty acids, which cause increased ceramide accumulation, activate inflammatory pathways, and increase the release of reactive oxygen species (ROS) and enhance apoptosis.[32]

Oxidative stress

Oxidative stress is central to the development of insulin resistance and diabetic complications.[33–34] Oxidative stress plays a critical role in diabetic complications. Hyperglycemia leads to the overproduction of superoxides in mitochondria. This increase in superoxide production activates several pathways that contribute to diabetic complications, including polyol pathway flux, increased AGE formation and RAGE expression, and activation of protein kinase C and the hexosamine pathway.[18] Inflammation induced by increased intracellular ROS also contributes to diabetic complications.[35] After ROS, are created, they deplete cellular antioxidant defences, rendering the affected cells and tissues more susceptible to oxidative damage.[36] It has been shown that ROS not only play a role in the destruction of cells and tissues but also function as intracellular second messengers that regulate signal transduction cascades and gene expression. Oxidative stress can also induce the activation of multiple serine kinases, which impair the capacity of insulin to stimulate protein kinase B activation and glucose transport. NF-κB, p38 MAPK and the JNK/SAPK pathway are sensitive to oxidative stress, which is linked to impaired insulin action and the development of the late diabetic complications.[33]

Immune response

Neutrophils play a crucial role in several autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis. Some studies have noted the involvement of neutrophils in T1DM; a mild neutrophil reduction has been associated with T1DM subjects;[37–38] however, neutrophil counts in T2DM patients are normal.[37] The reduction in circulating neutrophils that is observed in T1DM might be due to impaired neutrophil differentiation and output from bone marrow, increased neutrophil apoptosis or anti-neutrophil-specific antibodies, and increased recruitment into tissues.[39] The functional alteration of mononuclear phagocytes has also been reported in diabetic subjects, including altered superoxide (O_2^-) production, defective chemotaxis, and phagocytosis.[40] A study has shown that infiltrating monocytes in T1DM subjects spontaneously secrete pro-inflammatory cytokines, which are known to induce and expand Th17 cells.[41] Evidence also shows that the classically activated macrophages initiate insulitis and β-cell death in T1DM subjects and play a role in insulin resistance in T2DM by triggering an inflammatory response. In contrast, alternatively activated macrophages exert a protective effect in DM by attenuating tissue inflammation.[42]

Pro-inflammatory factors

Adipose tissue appears to be a major site for inflammatory mediator production as a result of cross-talk between adipose cells, macrophages, and other immune cells that infiltrate the expanding adipose tissue.[43] Inflammatory mediators might play a dual role in T2DM, contributing to hyperglycemia-induced insulin resistance and contributing to diabetic complications.[44] Pro-inflammatory factors, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and IL-18, are reportedly increased in diabetes mellitus and contribute to insulin resistance by both JNK and the IKKβ/NF-κB pathway.[45–46] Enhanced production of inflammatory cytokines is thought to contribute to insulin resistance and the destruction of beta cells in the pancreas and is thought to be a major factor in the development of diabetic complications.[47–48]

Anti-inflammatory factors

The role of pro-inflammatory cytokines in the destruction of pancreatic β cells and the development of T2DM has been investigated; however, our knowledge about anti-inflammatory proteins is rather limited, and an imbalance between pro- and anti-inflammatory cytokines might be essential for the development of DM.[49–50] IL-1 receptor antagonist is a naturally occurring anti-inflammatory antagonist of the IL-1 family of pro-inflammatory cytokines.[51] Evidence shows that blocking IL-1β signals reduces the expression of inflammatory cytokines, and IL-1 receptor antagonist improves glycemic control and counteracts β-cell destruction.[52] Acting as a hormone with anti-inflammatory insulin-sensitizing properties, adiponectin reportedly associates with a decrease in the risk of T2DM.[53] However, increased circulating levels of adiponectin are found in T1DM patients, this might be explained by the dependence of T2DM on insulin resistance.[50]

COMPLICATIONS OF DIABETES

T1DM and T2DM have many possible long-term complications. Epidemiological studies indicate that the severity of diabetic complications is generally proportional to the degree of hyperglycemia.[54]

Macrovascular complications

The injurious effects of diabetes on the vascular system are traditionally divided into macrovasular and microvascular complications.[55] Evidence suggests that diabetic patients are three times more likely

to suffer cardiovascular events than are non-diabetic subjects.[56] Cardiovascular disease is the most common cause of death in diabetic patients.[57] The central pathological mechanism in macrovascular disease is atherosclerosis. Atherosclerosis is thought to result from chronic inflammation of the arterial wall in the peripheral or coronary vascular system. One mechanism is the stimulation of lipid oxidation of low-density lipoprotein in diabetes mellitus, which accumulates in the endothelial wall of arteries.

Microvascular complications

High serum glucose levels affect endothelial cells that line blood vessels and cause the basement membrane to become thicker and less effective.[58] Microvascular complications are a significant part of diabetic retinopathy, nephropathy and neuropathy.[59] Diabetes-enhanced TNF induces the loss of microvascular endothelial cells and pericytes by activating the transcription factor FOXO1.[60–61]

Retinopathy

Diabetic retinopathy is clinically classified into non-proliferative and proliferative disease stages. Only intraretinal microvascular changes are present in non-proliferative diabetic retinopathy, whereas new blood vessels are formed on the retina or on the optic disk in proliferative diabetic retinopathy.[62] Diabetic retinopathy is characterized by several common and unique features, including thickening of the vascular basement membrane, pericyte and endothelial cell death, microaneurysms, vascular occlusion and pathological neovascularisation, which advance to retinal haemorrhage, retinal detachment, and vision loss.[63] TNF has been shown to play an important role in stimulating the early events of diabetic retinopathy.[60]

Neuropathy

Diabetic neuropathy is characterized by progressive neuronal loss, demyelination, and impaired nerve regeneration, with ultimate nerve fibre dysfunction.[62] Diabetic neuropathy can affect sensory, motor and autonomic nerve fibres in any part of the body.[64] Although diabetic neuropathy has been widely studied for over 20 years, the pathogenesis of this disease remains unclear; diabetic neuropathy is thought to result from the diabetic microvascular injury of small blood vessels that supply nerves, as well as oxidative injury, AGEs and insulin deficiency.[65]

Nephropathy

Hyperglycemia induces cellular changes in various kidney cell types. Nephropathy is a progressive kidney disease that is caused by the angiopathy of capillaries in the kidney glomeruli and is characterized by glomerular hypertrophy, thickening of basement, tubular and glomerular membranes and the accumulation of extracellular matrix in these membranes; these changes finally cause tubulointerstitial and glomerular fibrosis and sclerosis.[66] AGEs, hyperglycemia and vascular inflammation are thought to cause these pathogenic changes.[67–68]

DIABETES-RELATED BONE DISEASES

In addition to the complications mentioned above, diabetes affect bone metabolism. A considerable amount of evidence has accumulated indicating that metabolic and endocrine alterations caused by diabetes affect bone quantity and quality over the last decades of life.[69]

Increased fracture risk

T1DM and T2DM are associated with higher fracture risk. A large case control study in Denmark reported a relative risk for any site fracture of 1.9.[70] The relative risk of hip fracture is estimated as 6.9 in T1DM patients and as 1.38 in T2DM patients.[71] Women with T2DM have a threefold higher risk of vertebral fracture compared to women who are non-diabetic.[72] In the women's health initiative study, the risk of proximal humerus, foot, and ankle fractures was found to be higher among women with type 2 diabetes than among healthy controls.[73]

Changed bone mineral density

Bone mineral density (BMD) is reduced in T1DM.[72,74] Decreased BMD and diabetic vascular complications are associated with an increased risk of fractures. In T2DM, BMD is equal or increased according a meta-analysis,[71] but the fracture risk is increased despite this increase in BMD.[75] The paradoxical increase in fracture rate in patients with T2DM with increased BMD might result from an increased rate of falling.[76] In addition, decreased bone formation with decreased bone quality is thought to contribute to a higher fracture risk in T2DM. Rodent models also support the hypothesis that diabetic bone has reduced mechanical strength, regardless of bone density.[77]

Lower bone turnover rate

Decreased bone turnover rate might explain the increased bone fragility in patients with diabetes. Older bone is not replaced by new bone with decreased bone turnover rate, thus decreasing bone mechanical strength. This is reflected in studies indicating that diabetic patients exhibit decreased osteoblast activity.[78] Low bone turnover accompanied by reduced mineral content was found in T1DM models.[79] Leptin receptor-deficient mice, a T2DM model, also exhibit decreased bone turnover.[80–81] The impact of diabetes on bone resorption has yielded contradictory findings, with some studies indicating increased osteoclast activity under perturbation.[82–83]

Delayed fracture healing

Fracture healing is delayed in diabetic patients. A study of 5 966 cases of hip fracture in patients with diabetes reported that these patients required longer in-hospital stays.[84] Moreover, diabetic patients undergoing ankle fusing procedures exhibit an increased incidence of non-union outcomes (from 27% to 38%).[85] In diabetic mice that were fed a high fat diet, fracture calluses were significantly smaller and had markedly increased adiposity.[86] In streptozotocin-induced diabetic mice, fracture calluses exhibited reduced bone formation, which was associated with premature cartilage resorption.[87]

POTENTIAL MECHANISMS BY WHICH DIABETES AFFECTS BONE

Bone formation and resorption affect bone, and diabetes mellitus affects both bone formation and resorption.

Effect of diabetes on osteoblasts

Diabetes has a significant effect on osteoblasts. It has consistently been noted that diabetes causes a reduction in the number of bone-forming cells.[88] One of the mechanisms through which diabetes affects osteoblasts is increased apoptosis. For example, AGEs induce osteoblast apoptosis through the MAP kinase pathway.[89] Diabetes also interferes with bone formation by reducing the expression of transcription factors that regulate osteoblast differentiation.[90] In rat models of T1DM and T2DM, osteoblasts exhibit lower alkaline phosphatase activity and mineralized matrix formation.[91–92] When AGEs are applied to wounds in normal animals, the rate of healing is reduced by half, suggesting that AGEs, which are present at higher levels in diabetes, contribute to impaired bone healing.[93] In addition, the receptor for AGEs, RAGE, is expressed at higher levels in osteoblasts in diabetic

conditions, thus rendering diabetic animals even more sensitive to the effects of AGEs.[93]

Inflammation also has a significant effect on bone.[94] Elevated levels of inflammatory mediators, particularly TNF, are among the striking features of diabetes.[95] Diabetic animals exhibit higher levels of TNF in bone, and enhanced TNF levels are associated with reduced bone healing. Thus, diabetes-enhanced TNF contributes to an increase in bone cell apoptosis, which reduces osseous healing.[96] Moreover, diabetes causes a more than two-fold induction of more than 70 genes that directly or indirectly regulate apoptosis during inflammation-induced bone injury and that significantly enhance caspase-8, -9 and -3 activity.[97] A significant effect on bone, which was induced by cell death in diabetic animals, was shown by treating mice with a pancaspase inhibitor. Treatment with this inhibitor increased the number of bone-lining cells and enhanced new bone formation.[97] Profiling of mRNA expression during diabetic fracture indicates that gene sets that are related to apoptosis are significantly upregulated.[98] The increased expression of apoptotic genes is associated with elevated TNF-α levels, increased chondrocyte apoptosis and increased activity of the pro-apoptotic transcription factor, FOXO1. Inhibition of TNF significantly reduces each of these effects in diabetic fracture healing but has little effect in normoglycemic mice.[98]

Mesenchymal stem cells represent a precursor pool of osteoblasts, which are bone-forming cells. Inflammation, which is elevated in diabetic bone healing,[96] has a significant effect on reducing mesenchymal stem cell (MSC) differentiation.[99] Inflammation affects MSC through the induction of NF-κB activation. Increased NF-kB activity interferes with wnt-stimulated MSC differentiation by increasing beta-catenin degradation.[100] Moreover, TNF suppresses Osx promoter activation,[101] thus interfering with the differentiation of MSCs to osteoblasts because osterix is required during the early steps of differentiation. AGEs also inhibit MSC differentiation. One mechanism by which this occurs involves the upregulation of ROS by AGEs in MSCs, leading to a decrease in MSC differentiation.[102–103] In human mesenchymal stem cells and mouse stromal ST2 cells, AGEs suppress the osteogenic differentiation of both cell types by increasing TGF-β expression.[104] In a T2DM mouse model, the diabetic mouse has fewer MSCs, and these MSCs exhibit poor ability to target the injury site.[105] In a T1DM rat, more numerous apoptotic cells were present in hyperglycemic bone marrow, and the size of the osteoprogenitor pool was significantly reduced.[92] Thus, elevated levels of TNF and the effects of AGEs might interfere with the production of osteoblasts that are formed by inhibiting the differentiation of MSC to osteoblasts.

Effect of diabetes on osteoclasts

Human studies of diabetes mellitus generally indicate that osteoclastogenesis is enhanced. Humans with T2DM exhibit increased circulating levels of tartrate-resistant acid phosphatase, which is indicative of increased osteoclast activity.[82] In patients with T1DM or T2DM, poor glycemic control leads to increased bone resorption and bone loss.[78] In patients with T2DM, levels of the bone resorption marker tartrate-resistant acid phosphatase are increased in serum, indicating increased osteoclastic function, which might be due to the diabetic polyol pathway.[106] However, some studies indicate that bone resorption markers are decreased in individuals with T2DM.[107]

Animal studies generally indicate that osteoclast activity is increased in diabetic patients.[108–109] In streptozotocin-induced T1DM rats, cathepsin K expression is elevated compared to a control group, indicating increased osteoclast activity.[110] In T2DM rats, osteoclastic bone resorption is enhanced compared to normoglycemic controls.[111] In diabetic mice, TNF-α, macrophage-colony stimulating factor, receptor activator of nuclear factor kappa-B ligand (RANKL) and vascular endothelial growth factor-A are upregulated; this upregulation can directly promote osteoclast differentiation and activation.[87,112] Patients with T2DM exhibit elevated mitochondrial ROS levels, which promote RANKL-mediated osteoclast differentiation and function.[113] Increased fatty acid levels in patients with diabetes mellitus can induce osteoclastogenesis by TNF-α.[114] T2DM mice exhibited enhanced osteoclast formation in response to M-CSF and RANKL.[82] *In vitro* studies suggest that hyperglycemia predisposes patients to increased osteoclast formation.[115] AGEs might also increase osteoclast activity.[116] Mice that lack the receptor for AGE, RAGE, exhibit increased bone mass and decreased osteoclast numbers compared to wild-type mice,[117] supporting the concept that AGEs contribute to osteoclast formation in patients with diabetes.

PERIODONTAL DISEASE: INTRODUCTION

Periodontitis is one of the most widespread oral diseases and is characterized by the loss of connective tissues within the periodontium and the destruction of alveolar bone support.[118–119] Severe periodontitis, which can result in tooth loss, is found in 5%–20% of most adult populations worldwide. The latest data from the 2009 and 2010 National Health and Nutrition Examination Survey cycle estimates that over 47% American adults have had periodontitis.[120] An epidemiology study shows that almost 25% of adults in Australia aged 35–54 years have moderate or severe periodontitis, and 34% of 30- to 39-year-old adults living in Pomerania have periodontitis.[121] Children and adolescents can have any of the several forms of periodontitis, such as aggressive periodontitis, chronic periodontitis and periodontitis as a manifestation of systemic diseases.[122] However, estimates of the global prevalence of periodontitis differ based on the distribution of the disease and the methodologies used to assess it.[123]

PATHOGENIC MECHANISMS OF PERIODONTAL DISEASE

Microorganisms

The chronic inflammatory condition of periodontitis is induced by pathogenic biofilms or dental plaque, which accumulates on the tooth surface. Over 500 bacterial species have been detected in periodontal plaque; however, the composition of the causative bacterial species is still under debate.[123–126] 'Red complex' Gram-negative bacteria, comprising *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*, have been proposed as the primary aetiological agents of periodontitis.[127] Recent studies have identified *Filifactoralocis* and many species of *Spirochetes* as being closely linked to periodontitis.[128] As the best characterized periopathogen, *P. gingivalis* is a minor constituent of the total microbiota but can trigger changes in the amount and composition of the oral commensal microbiota that can disrupt homeostasis and cause inflammatory periodontal bone loss.[129] Gram-positive bacterial and oral commensal bacteria might also play a significant role in the development of periodontitis [128,130–131].

Modern high-throughput techniques indicate that the subgingival microbial profiles from periodontitis patients differ according to age, pocket depth, gender and race.[128,132–133] Interestingly, bacteria community diversity has been shown to increase with a history of periodontitis, which runs counter to most bacterial infectious processes, which are generally associated with decreased diversity.[128]

Host response

Although bacteria are necessary for periodontal disease to occur, a susceptible host is also needed.[123] The inflammatory process occurring

in periodontitis is characterized by the infiltration of leukocytes, which limit the level of bacterial invasion and can be harmful to the periodontal tissue.[134] Periodontal ligament and bone destruction is thought to be caused by a disruption of the homeostatic balance between the host response and bacteria, which causes inflammation in close proximity to bone.[129,134–135] The process is thought to involve the host immune response to bacterial infection through the stimulation of osteoclastogenic factor production by immune cells, which then help to cause the periodontitis-related bone loss. Our laboratory has recently shown that the production of factors by osteoblasts and osteocytes also contributes to osteoclast formation and activity in periodontal disease (unpublished data). Evidence that the host response plays a critical role has also shown by studies in which treatment with a prostaglandin inhibitor reduced periodontitis-related bone loss[136] and studies involving the inhibition of inflammatory cytokines, such as IL-1 and TNF.[137–138] Thus, periodontitis is a complex disease in which multiple causal risk factors play simultaneous and interactive roles; these risk factors include the immune-inflammatory status and genetic background of individuals, and the presence of environmental stressors and/or systemic diseases, such as diabetes.[139–141]

Effects of diabetes on periodontitis

Diabetes and chronic periodontitis are chronic diseases that have long been considered to be biologically linked.[142–143] In fact, diabetes is one of the primary risk factors for periodontitis.[144–145] Cross-sectional and longitudinal studies identified that the risk of periodontitis is approximately 3–4 times higher in people with diabetes than in non-diabetic subjects.[144] Periodontitis was found in 57.9% of T1DM patients and in 15.0% in controls without diabetes.[146] In another study on the periodontal status in children and adolescents with T1DM, a prevalence of 20.8% of gingivitis and 5.9% of periodontitis was observed.[147] Patients with T2DM were also at higher risk of having severe forms of periodontitis compared with non-diabetic subjects.[148] A study in African Americans found that 70.6% of patients with T2DM had moderate periodontitis, and 28.5% had a severe form of the disease; this value is significantly higher than the prevalence of 10.6% among control subjects without diabetes.[149] A direct relationship exists between the level of glucose control and the severity of periodontitis.[146,150] The odds ratios of T2DM with periodontal destruction in comparison to non-diabetics are 1.97, 2.10 and 2.42 in well, moderately and poorly controlled diabetics, respectively.[151]

EFFECTS OF DIABETES ON PERIODONTIUM TISSUES

Gingiva/gingivitis

Periodontitis is preceded by various stages of gingival inflammation, which are referred to as gingivitis. The prevalence of gingivitis in children and adolescents with T1DM is nearly twice that observed in children and adolescents without diabetes.[152] Evidence shows that the gingival index was 1.54 in a 5- to 9-year-old diabetic group and 1.14 in the control group; however, in the corresponding 10- to 14-year-old group, the gingival index was 1.98 in diabetic subjects and 1.17 in control subjects.[153] Furthermore, gingival bleeding index is significantly correlated with age and the level of glycosylated haemoglobin.[154] Similarly, the rates of gingival inflammation in adults with T2DM are higher than those in adults without diabetes. Nearly 64% of patients with T2DM have gingivitis; however, only 50% of subjects without diabetes have the disease.[152] The degree of metabolic control of diabetes is an important factor in the development and progression of gingivitis; good control significantly reduces the prevalence of gingivitis.[155–156] In animal models, diabetes leads to the increased production of TNF in the epithelium and connective tissue.[157] Periodontal infection causes an increase in epithelial cell and connective tissue fibroblast apoptosis, which is significantly enhanced by diabetes through a caspase-3-dependent mechanism.[157] This is significant because increased diabetes-enhanced inflammation and apoptosis are thought to negatively impact the gingiva by causing a loss of epithelial barrier function and inhibiting repair processes.[158–159] Animal studies have demonstrated that high levels of TNF-α can stimulate the expression of pro-apoptotic genes, which induce apoptosis.[96,160] *In vivo* studies have demonstrated that the inhibition of TNF-α reduces connective tissue cell apoptosis.[161]

Periodontal ligament/loss of attachment

Periodontitis involves the loss of tooth-supporting structures comprising connective tissue attachment and bone. A statistically significant association is observed between diabetes and loss of attachment, and the level of glycemic control is an important determinant of this relationship.[146,162] The odds ratio of T1DM patients with attachment loss is 3.84 compared with non-diabetic subjects.[163] In addition, more than 25% of T1DM patients with poor metabolic control exhibit sites with clinical attachment loss of $\geqslant 5$ mm, compared with 10% of subjects with good metabolic control.[152] Subjects with T2DM also exhibit a significantly higher number of sites with clinical attachment loss of $\geqslant 3$ mm as well as loss of $\geqslant 5$ mm. Furthermore, the prevalence of sites with moderate to severe attachment loss is proportional to the duration of diabetes.[150,164]

Alveolar bone/loss of bone

Diabetes potentiates the severity of periodontitis and accelerates bone resorption. The percentage of sites with bone loss in poorly controlled T1DM individuals is 44% compared to 28% and 24% in well-controlled and non-diabetic subjects, respectively.[165] Animal studies also demonstrate that alveolar bone loss in rats with STZ-induced T1DM with periodontitis was threefold higher than in normal rats.[166–167] The risk and degree of alveolar bone loss is positively correlated with lack of metabolic control.[168–169]

MECHANISMS OF DIABETES ENHANCED PERIODONTAL BONE LOSS

The process of bone remodelling starts with the resorption of bone by osteoclasts, followed by new bone formation by osteoblasts in the resorption lacunae. Under physiological conditions, these two activities are coupled; however, the two processes are uncoupled in pathological processes.[118,134] Diabetes affects osteoclast and osteoblasts in the periodontium in different ways, such as by increasing the expression of inflammatory mediators and RANKL/osteoprotegerin (OPG) ratios and by enhancing the levels of AGEs and ROS (Figure 1).

Effect of diabetes on osteoclasts in periodontitis

Diabetes has been shown to enhance osteoclast formation in inflamed areas. Rats with T2DM exhibit a two- to four-fold increase in osteoclast number after bacterial infection by oral inoculation of a periodontal pathogen ligature that induces periodontitis compared with control rats.[111,157,170] Rats with T1DM and periodontitis also exhibit a two- to four-fold increase in the number of osteoclasts compared to non-diabetic rats with periodontitis.[171]

A higher degree of inflammation and a more persistent inflammatory response following periodontitis are reported in rats with T1DM and T2DM in response to the same inoculation of periodontal pathogen.[172–173] Diabetes impairs the resolution of periodontal

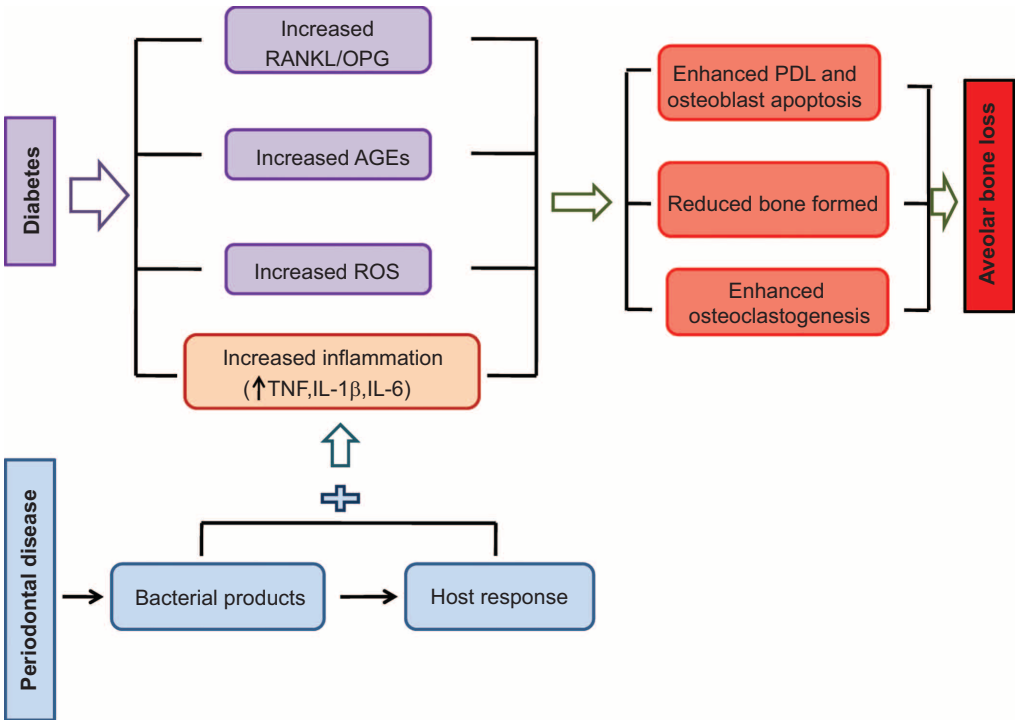

Figure 1 A potential mechanism of diabetes-related alveolar bone loss in periodontal disease. Diabetes increases the RANKL/OPG ratios and enhances the expression level of AGEs, ROS and inflammatory mediators, which induce osteoblast apoptosis and osteoclastogenesis. This cascade of events contributes to both increased bone resorption and reduced reparative bone formation leading to greater alveolar bone loss in periodontal disease that is caused by bacterial pathogens. AGE, advanced glycation end product; IL, interleukin; OPG, osteoprotegerin; PDL, periodontal ligament; RANKL, receptor activator of nuclear factor kappa-B ligand; ROS, reactive oxygen species; TNF, tumor necrosis factor.

inflammation. The importance of resolving inflammation has been demonstrated by treating animals with periodontitis with resolvins[174] or by treatment of diabetic animals with TNF inhibitors.[160,170] Diabetes-enhanced TNF has been shown to prevent the downregulation of genes that are associated with host defence, apoptosis, cell signalling and activity, and coagulation/homeostasis/complement.[175] Similarly, patients with periodontitis and diabetes were found to have significantly higher levels of local inflammatory mediators such as IL-1β, TNF-α and prostaglandin E_2, which result in more prolonged osteoclast formation and activity.[176] Enhancement of IL-17 and IL-23 in periodontitis in subjects with T1DM and overexpression of IL-1β and IL-6 in patients with T2DM have been reported; and these conditions result in osteoclastogenesis and a prolonged duration of inflammatory responses.[177–178] Patients with T2DM and periodontal disease exhibit increased levels of TNF-α and IL-6, which are also associated with increased dyslipidemia and lipid peroxidation.[179] These results suggest that there is an important association between T2DM, dyslipidemia and the severity of the local inflammatory response to bacteria.[179]

The interaction of RANKL with receptors (RANK) on the surface of osteoclasts is one of the most potent inducers of osteoclast formation and activity, and OPG inhibits osteoclast formation binding to RANK and then blocks the activity of RANKL.[169–170,180] A number of studies focusing on osteoclastogenesis-related factors have reported an elevated expression of RANKL and TNF in diabetes-associated periodontal tissues.[180] Studies with animals suggest that RANK-RANKL/OPG ratios and the level of other inflammatory cytokines, such as TNF, are critical mediators of the enhanced osteoclastogenesis in diabetes with periodontal disease.[170–171,181] TNF levels and the RANKL/OPG ratio in periodontitis sites in humans are negatively influenced by poor glycemic control in subjects with diabetes.[179,182]

Diabetes enhances the formation of AGEs in the periodontium and increases the expression of RAGE.[183] Gingiva AGEs are increased in both T1DM- and T2DM-associated periodontitis; however, there is evidence that subjects affected with T1DM presented a significantly higher percentage of AGE-positive cells in the epithelium and fibroblasts than did subjects affected with T2DM.[184] Osteoclast-like cells express RAGE, which serves as a positive factor that regulates osteoclast formation.[117] AGE accumulation and the interaction of AGEs with RAGE may contribute to osteoclastogenesis via an increased expression of the receptor activator of RANKL and OPG downregulation.[185] It has been shown that the AGE-RAGE interaction on monocytes activates transcription factor NF-κB, which alters the phenotype of monocytes/macrophages and results in the increased production of pro-inflammatory cytokines.[186]

ROS are known to be one of causes of diabetes-related periodontitis. The invading bacteria trigger the release of inflammatory cytokines, leading to increases in the number and activity of neutrophils, which release ROS in periodontitis.[187] During bone resorbtion, osteoclasts that contain NADPH-oxidase actively produce superoxide.[188] Simultaneously, neutrophils in diabetic patients produce more superoxide than neutrophils from normal subjects.[26] The imbalance between ROS production and antioxidant defences result in increased oxidative stress.[189] In addition, the formation of AGEs also increases oxidative stress in periodontium tissue. It has been shown that certain ROS (such as superoxide and hydrogen peroxide) activate osteoclasts and promote osteoclast formation.[190] A related process, lipid peroxidation, is also associated with increased periodontal disease-T2DM

and a greater inflammatory response in periodontal tissues in humans.[179,191]

Effect of diabetes on osteoblasts in periodontitis

Evidence exists that both diabetes and bacterial infection in periodontitis enhance the apoptosis of osteoblastic cells, thereby reducing osseous coupling.[161,192] It is likely that both the innate and adaptive immune responses that are induced by bacterial infection cause osteoblastic cell loss.[96,193] Diabetes also increases the loss of periodontal ligament (PDL) cells that is induced by periodontal infection by increasing the apoptosis of these cells.[111,194] This loss is significant because the PDL is a rich source of cells that are capable of differentiating into osteoblasts. Studies in diabetic animals indicate that diabetes causes a more than 2-fold induction of genes that regulate the apoptosis of osteoblasts and fibroblasts following bacterial infection and a fivefold increase in osteoblast apoptosis.[97,195] The apoptosis of osteoblasts is a significant component of the failure of diabetic animals to form new bone after the induction of periodontal disease, as shown by a significant increase in bone formation when diabetic animals are treated with a specific blocker of apoptosis following periodontal infection.[161]

Diabetes leads to the upregulation of pro-apoptotic factors for osteoblasts, including advanced TNF-α, AGEs and the formation of ROS, each of which can contribute to apoptosis.[161] Animals with T1DM and T2DM exhibit higher levels of TNF-α in response to a bacterial stimulus than normoglycemic controls.[170,184] Enhanced TNF-α levels have been directly linked to cellular changes in diabetes-enhanced periodontitis.[196] TNF-α impairs the function of osteoblasts by blocking the differentiation of osteoblasts when inflammation is thought to be present. Furthermore, TNF-α can induce apoptosis by binding to TNF receptor-1, which triggers the initial events in apoptosis.[158]

It has been shown that increased bacterial infection-induced alveolar bone loss in diabetic subjects is accompanied by the enhanced expression of RAGE and inflammatory AGEs in gingival tissue.[197] AGEs have been shown to interfere with osteoblast differentiation and to induce the apoptosis of osteoblasts in diabetes via the mitogen-activated protein kinase and cytosolic apoptotic pathway.[89] Elevated levels of AGEs are found in the periodontium of diabetics, and the AGE–RAGE interaction results in an increase in pro-inflammatory cytokine expression and induces osteoblast apoptosis.[186,198–199] CML-collagen, an AGE that is found in bone and serum, stimulates the apoptosis of bone-lining cells *in vivo* and in various osteoblastic cell cultures, which is mediated by RAGE.[200]

ROS production is another mechanism by which diabetes increases apoptosis. Persistent inflammation and hyperglycemia leads to cellular ROS accumulation, which is linked to diabetic complications.[199,201] Moreover, increased oxidative stress in periodontal tissue has been shown to induce osteoblast apoptosis.[202] It has been demonstrated that ROS causes the activation of caspase-3,[203] which mediates osteoblast apoptosis.

CONCLUSION

In summary, diabetes mellitus and periodontal diseases are closely associated and exhibit similarities to other chronic diseases. Persistent hyperglycemia leading to exaggerated immune-inflammatory responses that are induced by periodontal pathogens is likely to be responsible for the greater risk and severity of periodontal disease in diabetics.[134,199] Moreover, severe periodontitis that results in alveolar bone loss is likely to involve the effect of inflammation on both osteoclasts and osteoblasts. Diabetes has an important effect on enhancing osteoclastogenesis and on increasing osteoblast apoptosis. Interestingly, the effect of diabetes on bone loss and coupled bone formation is likely to involve the impact of diabetes on both the innate and adaptive immune responses.[47,134]

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