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REVIEW ARTICLE

Effect of inflammation on bones in diabetic patients with periodontitis via RANKL/OPG system-A review

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Abstract

Purpose Diabetes mellitus and periodontitis are inflammatory diseases, the severity of inflammation results in the progression and persistence of both the disorders and affects bones. Diabetic complications aggravate in diabetic subjects having periodontitis; similarly, diabetic patients are more prone to developing gingivitis and periodontitis. Periodontal and diabetic inflammation disturbs bone homeostasis, which possibly involves both innate and adaptive immune responses. The pathogenic processes that link the two diseases are the focus of much research and it is likely that upregulated inflammation arising from each condition adversely affects the other. RANKL/OPG pathway plays a prominent role in periodontal and diabetic inflammation and bone resorption.

Method This review article summarises the literature on the link between inflammatory cytokines and the prevalence of disturbed bone homeostasis in diabetic patients with periodontitis. An extensive search was done in PubMed, Scopus, Medline and Google Scholar databases between April 2003 and May 2021.

Result A total of 27 articles, including pilot studies, case–control studies, cross-sectional studies, cohort studies, randomized control trials, longitudinal studies, descriptive studies and experimental studies, were included in our literature review.

Conclusion Since RANKL/OPG are cytokines and have immune responses, regulating these cytokines expression will help control diabetes, periodontitis and bone homeostasis. The growing evidence of bone loss and increased fracture risk in diabetic patients with periodontitis makes it imperative that health professionals carry out planned treatment focusing on monitoring oral health in diabetic patients; bone markers should also be evaluated in patients with chronic periodontitis with an impaired glycemic state.

Keywords Inflammation · RANKL/OPG · Bones · Diabetes · Periodontitis

Abbreviations

T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
RANKL	Receptor activator of nuclear factor-kappa B – Ligand
OPG	Osteoprotegerin
NF- κB	Nuclear factor-kappa B
IL1-β	Interleukin 1 beta
IFN-γ	Interferon Gamma
TNF-α	Tumor Necrosis Factor-alpha
PG-E2	Prostaglandin-E2
TGF-β	Transforming Growth Factor beta
Runx-2	Runt related transcription factor 2
AGEs	Advanced glycation end products
RAGE	Receptor-AGE
R0S	Reactive oxygen species

Introduction

Diabetes mellitus (DM) and periodontitis are chronic inflammatory diseases linked biologically, as evident by many studies and remarkably impact human health [1]. The interconnection between the prolonged activated immune system and skeletal deterioration spawned the emergence of osteoimmunology [2]. Systemic inflammation also affects bone homeostasis; however, there is a paucity of information on the effect of diabetic and periodontal inflammation on bones.

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This review focuses on how diabetes and periodontitis impact bone homeostasis by regulating RANKL/OPG levels. The information presented in this review was searched from available literature in PubMed, Scopus, Medline and Google Scholar databases between April 2003 to May 2021. Systemic searches methods using a combination of search terms explored abstracts of the published articles and other articles were searched manually from the citations. The search keywords entered in the databases included diabetes and/or periodontitis, bone health, bone loss, inflammation, RANKL/OPG, periodontitis and/or bone health, bone loss, inflammation, RANKL/OPG. A total of 27 articles were scrutinized, including pilot studies, case-control studies, cross-sectional studies, cohort studies, randomized control trials, longitudinal studies, descriptive studies and experimental studies. This paper discusses the role of inflammatory cytokines and RANKL/OPG in the pathogenesis of diabetes, periodontitis and bone resorption from the available research database and provides an update on how the resolution of inflammation and non-surgical periodontal therapy improves bone health.

Effect of diabetic inflammation on periodontitis-

A dysregulated immune system marked by changes in cytokine levels is one of the crucial factors in the pathogenesis of diabetes with a long-lasting inflammatory state. [3]. The activated innate immune system further leads to the destruction of insulin-producing beta (β) cells [4]. Impaired insulin secretion due to the destruction of β cells in type 1 diabetes mellitus (T1DM) and incompetent expression of glucose transporter (GLUT)-2 in type 2 diabetes mellitus (T2DM) contribute to hyperglycemia. As a result, the pro-inflammatory transcription factor nuclear factor kappa B (NF-Kb) is activated [5]. Levels of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1 β, IL-6 and IL-10 further contribute to insulin resistance induced due to hyperglycemia and enhance diabetic complications [6]. The neutrophils cannot function normally; the chemotactic activity is reduced as well as the phagocytosis and bactericidal activity are also decreased [7]. The increase in glucose concentration leads to increased cellular stress; and as a result, glycation end products are formed [8]. The generated reactive oxygen species (ROS) in mitochondria enhance the expression of TNF-α [9]. All these conditions contribute to the exacerbation of inflammation in diabetic patients.

The last several decades have witnessed a rapid rise in the number of cases of DM and is predicted to be the seventh leading cause of death by 2030, as reported by WHO [10, 11]. DM is a significant contributing factor for the pathogenesis of periodontitis [12]. Hyperglycemic patients are more likely to suffer from periodontitis; similarly, periodontitis patients have difficulty in controlling serum glucose [13]. Prolonged inflammation and dysregulation of cytokine networks in diabetes upregulate genes that regulate host response and apoptosis regulating genes in the periodontium resulting in elevated inflammation during resolution of periodontitis [14]. Due to the excess accumulation of glucose in periodontal tissue, the blood vessels are damaged, and the regeneration of collagen structures is reduced [15]. Thus there is a plausibility that elevated and prolonged inflammation is a prominent factor that makes diabetic patients more prone to periodontitis.

Effect of periodontal inflammation on diabetes

Periodontitis is mediated by diverse microbial flora and its numerous bacterial products [16]. However, immune responses have a crucial role in the pathogenesis of periodontitis [17]. Persistence inflammation in periodontitis increases the risk of several chronic diseases [18]. The presence of systemic diseases, especially diabetes, further causes an exaggerated immune response via microbial factors such as endotoxins resulting in enhanced periodontal tissue breakdown [19].

In DM, aggressive periodontitis has been reported as the sixth complication; conversely, periodontitis is said to be a risk factor for worsening glycemic control and aggravating diabetic complications, the prevalence of periodontal disease increases in both T1DM and T2DM, as evident by systematic reviews and meta-analysis [1, 12, 20, 21]. Cross-sectional studies support the association of periodontal inflammation and DM and reported resolution of inflammation from periodontal tissue improves HbA1c level after periodontal treatment [22, 23]. The periodontal tissues acknowledge increased cellular apoptosis due to reduced proliferation and potential to repair during enhanced diabetic inflammation [24]. Inflammation and oxidative stress can be sought as crucial regulators of the manifestation of diabetes and periodontitis [25].

Cross-sectional and longitudinal studies have reported a 3-4 fold increase in the risk of periodontitis in people with diabetes than in non-diabetic subjects as periodontal disease further aggravates the poor glycemic condition and diabetes-related complications [26, 27]. Several studies have reported increased levels of inflammatory markers such as IL-1β, TNF α and IL -6 in gingival crevicular fluid (GCF) of patients with diabetes and periodontitis [28, 29]. Levels of inflammatory mediators elevated in periodontitis prevent downregulation of inflammation [24]. Inflammatory cytokines such as TNF α and IL 6 are reported to promote insulin resistance. TNF α phosphorylates insulin receptor IRS-1 at serine residue; as a result, insulin receptor (IR) tyrosine kinase is impaired [5]. Elevated levels of IL-6 in combination with IL6/R phosphorylates AMPK and enhance subsequent glucose uptake in human skeletal

muscles independent of insulin [30]. In addition to this, advanced glycation end products (AGEs) accumulated in the periodontium as a result of hyperglycemic state affect the migration and activity of phagocytic mononuclear and polymorphonuclear cells; as a result, pathogenic subgingival flora flourishes, exacerbating the periodontal damage and loss of alveolar bone due to loss of gingival fibroblast synthesis [31]. Randomized control trial by Repone et al., 2021 supports the link between diabetes and periodontitis and how periodontal inflammation negatively affects glycemic control [32]. Early identification and treatment of diabetes can interrupt the progression of the disease, diabetic screening during dental visits is a promising approach to public health. Juan Wu et al., in their pilot study, have shown gingival crevicular blood can be used to measure blood glucose and HbA1c level in periodontitis patients during dental visits to identify at risk patients and optimize treatment at the correct time [33].

Regulation of RANKL/OPG pathway in bone homeostasis-

Skeletal growth postnatally and bone remodelling are highly coordinated processes primarily mediated by osteoblasts and osteoclasts [34, 35]. In addition to providing skeletal strength and protection to vital organs, bone is a mineral reservoir for calcium and is also a site of immune cell development [36, 37]. Osteoclasts are large multinucleated cells that digest the bone mineral and break down bone tissue; they act as a proton pump and create acid compartments resulting in tartrate-resistant acid phosphatase (TRAP) protease release, which degrade inorganic and bone components [38]. Bone forming osteoblast cells regulate osteoclastogenesis and orchestrate bone resorption by synthesizing bone matrix proteins and regulating mineralization [39].

Receptor activator of nuclear factor kappa-B (RANK) ligand (RANKL), its receptor RANK and osteoprotegerin (OPG) belong to the TNF family. RANKL is expressed by osteoblasts, fibroblasts and activated T and B cells [40]. OPG expressed by endothelial cells, vascular muscle cells and osteoblast is a cytokine that acts as an osteoclastogenesis inhibitory factor. Osteoprotegerin ligand (OPGL) regulates osteoclast differentiation and activation [4]. RANKL signalling is vital for the proliferation and differentiation of osteoclasts [41]. Bone homeostasis is widely regulated by the RANKL/OPG complex as the binding of RANKL to OPG instead of RANK inhibits bone resorption. OPG/RANKL are cytokines, thus potentiating the role of RANK/RANKL/OPG in the immune system and metabolism of bone [42]. The OPG/RANKL system works analogously to the interleukin-cytokine system [43].

Osteoblast cells produce OPG, which functions as a paracrine inhibitor of osteoclast formation by binding to RANK [44]. Expression of RANKL is upregulated by osteoblast after osteocytes direct osteoclast to the site of bone resorption [45]. Dysregulation of RANKL signaling results in excessive or impaired bone resorption [46].

Inflammation is a major cause of bone homeostasis imbalance [47]. The concentration of OPG and RANKL for osteoclast differentiation is regulated by lysosomal cytokines, such as IL-1 β, IL-17, TNF-α; they stimulate osteoclastogenesis by reducing OPGL expression [2].

Effect of diabetes on bones-

DM is a metabolic disorder that has been reported to have increased fracture risk; bone formation is impaired, leading to delayed fracture healing [48]. Cohort studies related that fracture risk increases in DM patients, which can be due to upregulated inflammatory cytokines such as IL-21, T-regulatory cells and CRP levels [49–53]. Poor bone mineralization, crosslinking of collagen, increased expression of diabetogenic factor matrix metalloproteinase (MMP)- 9, decreased adiponectin level and upregulated pro-inflammatory cytokine levels are evident in T1DM and T2DM, resulting in reduced bone strength and increased fracture risk [54, 55].

Microvascular complications such as neuropathy, retinopathy, HbA1c level >8%, and decreased bone mineral density due to low bone turnover rate are possible links to increased fracture risk in diabetes [56]. Diabetic conditions such as hyperglycemic state, increased glycation of proteins, enhanced production of reactive oxygen species (ROS), and expression of inflammatory cytokines are the possible factors affecting bones in diabetes; however, the exact mechanism is unknown [48].

Effect of diabetes on bones due to increased glycation of proteins:

Hyperglycemic state leads to increased glycation of proteins [57]. The binding of AGES to the receptor-AGE (RAGE) leads to increased production of inflammatory cytokines NF-κB, which increases the production of RANKL and promotes osteoclastogenesis [58]. RAGE also affects osteoblast differentiation, marked by decreased concentration of bone alkaline phosphatase, collagen-1α-1, reduced bone matrix formation, and increased apoptosis of osteoblast cells [59]. Case-control studies have reported hyperglycemic state is responsible for loss of skeletal tissue in a diabetic condition, influencing the bone turnover markers, bone minerals and vitamin D [60]. Another-cross sectional study reports the negative impact of the hyperglycemic state on bone turnover markers [61].

Effect of diabetes on bones due to ROS-

Increased glucose concentration is responsible for the generation of superoxides by mitochondria which are also supported by increased glycated proteins, together with enhanced inflammation affect bones [9, 62, 63]. ROS negatively affect bones as they activate osteoclast activity by activating RANKL, which in turn negatively regulates forkhead box O (FoxO), leading to accumulation of ROS, thus enhancing bone resorption [64–66]. ROS also activate RAGE production to affect bone resorption via RANKL [67]. Decreased antioxidants in diabetic patients contribute to bone resorption, as differentiation of osteoclasts leads to more production of H_2O_2 concentrations [68].

Effect of diabetes on bones due to inflammation-

Of all the mechanisms affecting bone homeostasis in diabetic patients, it can be assumed that the inflammatory state has a more potent role, as the other pathways ultimately lead to upregulated inflammation. The elevated level of pro-inflammatory mediators such as TNF-α, IL-1 β, IL-6 IL-18 in diabetic patients and IL-1 β, TNF α, prostaglandins (PG)-E2 in diabetic patients with periodontitis promote an increase in levels of NF-κB and RANKL to promote osteoclast-mediated resorption of bones and decreased expression of runt-related transcription factor (Runx) – 2 the master transcription regulator that regulates bone formation [4, 34, 69, 70]. Increased osteoclastogenesis and reduced osteoblast activity result in enhanced caspase-3 activity, an enzyme that helps in osteoclast differentiation in T1DM and pro-apoptotic genes in T2DM [71]. The number of bone-forming cells is significantly reduced in diabetes, thus affecting bones [72].

Effect of periodontitis on bones due to inflammation-

In an inflammatory bone disease such as periodontitis, cytokines are released locally and systemically [25]. Upregulated inflammatory responses in DM increase the risk and severity of diabetic periodontitis and chronic inflammatory bone loss [73]. Since TNF-α limits the capacity to downregulate inflammatory genes, it upregulates apoptosis; as a result, bone homeostasis is disturbed by the increased osteoclast activity and increased bone resorption [74]. There is loss of connective tissue and bone in periodontitis, and significant attachment loss occurs in diabetes; thus, a relationship between glycemic control in diabetes and periodontitis with attachment loss and loss of bone can be drawn [22]. Under physiological conditions, RANKL produced by lymphocytes may not be involved in bone resorption; however, in pathological inflammatory states, increased concentration of serum RANKL mediates bone resorption [75]. Previous studies relate that increased concentration of RANKL and decreased concentration of OPG has been observed in gingival crevicular fluid and gingival tissues speculating that levels of RANKL play a significant role in bone resorption in periodontitis [76–78]. In a cross-sectional study by Sarlati *et al.,* 2012 RANKL concentration was higher in patients with moderation periodontitis and lowered OPG concentration in periodontal sites speculating loss of alveolar bone due to elevated sRANKL [79]. Cellular infiltrates of B cells, T cells and macrophages increases, thus affecting bone resorption by interacting with osteoblasts periodontal ligament. Increased expression of pro-apoptotic genes in T2DM also affects bones [80]. However, the exact mechanisms of alveolar bone resorption by local immune response has not been established yet [75]. Accumulation of AGEs in the periodontal tissues, increased secretion of cytokines such as TNF α, IL 1 β, IL 6, increased oxidative stress, disruption of NF-κB mediated RANKL/OPG axis, all conditions favour local tissue damage, periodontal connective tissue breakdown and bone absorption [15]. In diabetes and periodontitis, osteoclastogenesis is enhanced as the level of bone resorption markers increase leading to increased bone resorption and bone loss [48].

Moreover, downregulation of osteoblasts and inhibited downregulation of inflammation are possible reasons for disturbed bone homeostasis in diabetic patients with periodontitis. RANK/RANKL OPG signalling axis has been identified as an important regulator of bone remodeling; RANKL/OPG can be developed as promising biomarkers to identify high-risk patients having diabetes and periodontitis in the pre-clinical stage [44]. This enhances our need to improve our understanding of the mechanisms behind the development of bone complications in diabetic patients with periodontitis.

Conclusion

OPG/RANKL system regulates osteoclastogenesis and bone resorption. Since the system is upregulated by inflammatory mediators, which are common in diabetic patients with periodontitis, the diabetic and periodontal inflammation affects bone homeostasis and speeds up bone resorption. It is hard to evaluate the best method to apply for in vitro studies to assess the effect of glycemic control and reduced periodontal inflammation on bone density and alveolar bone loss as there are many inflammatory cytokines and oxidative parameters involved. Our review has not discussed the role of confounding factors such as supplemental vitamin D in improving bone health in the presence of diabetes and periodontitis. Studies correlating obesity, oxidative stress mechanisms and neutrophil-mediated osteoclastic bone resorption to

the progression of periodontal and diabetic inflammation and bone-related disorders were not included. It is essential to encourage further prospective studies to investigate the interrelationship between oral pathogenic bacteria and chronic systemic inflammation in diabetes and periodontitis with bone metabolism. There was only one pilot study that screened gingival crevicular blood to measure the presence and severity of diabetes through blood glucose and HbA1c level. Large case-control studies and randomized controlled trials are needed to validate if gingival crevicular blood can act as a precursor for the onset of diabetes.

It is implicated that multidisciplinary care centres in the context of diabetes, periodontitis and bone health should be set up. Medical and dental health care professionals keeping in mind the bidirectional relation between diabetes and periodontitis, should aware the patients about the existing link and carry out proper treatment. It should be advised that a patient with diabetes visits the dentist for an oral health check-up; similarly, dentists should enquire history of glycemic control, assess the risk of diabetes and any signs related to bone disorders. Organized research needs to be carried out on the assessment of OPG/RANKL concentration and other biomarkers of bone resorption in diabetic patients with periodontitis and with close monitoring of common inflammatory mediators and testing periodontal therapy efficiency in controlling inflammation and bone loss. Well, planned treatment strategies focused on managing diabetes and periodontitis involving surgical, non-surgical periodontal therapy and then assessing the improvement in bone homeostasis with organized research on osteoimmunology of bones together with diagnostic therapeutics, reducing the effect of inflammation on bones by the administration of OPG or upregulating OPG mediators promise good results in future. Figure 1

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Declarations

Conflict of Interest The authors report no conflict of interest related to the study.

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