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Review

Osteoprotegerin and RANKL Levels of Gingival Crevicular Fluid in Periodontal Disease

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Periodontal disease is an immunoinflammatory disease of the teeth-supporting tissues (the periodontium, i.e.).[1] Periodontitis is distinguished by bleeding or swollen gums (gingivitis), pain, and, occasionally, bad breath. In its most severe form, the gum can separate from the tooth and supporting bone, causing teeth to become loose and, in extreme cases, fall out.[2,3] Severe periodontal diseases are thought to affect nearly 10% of the world's population. "Periodontal disease is primarily caused by poor oral hygiene and tobacco use'' says WHO (World Health Organization).[3] It is a complex condition with a known multifactorial etiology. A bacterial infection causes an inflammatory response in the periodontal tissues. Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, and Treponema denticola are the most common periodontal pathogens.[4] Periodontal pathogens lead to the progressive deterioration of the periodontal ligament and alveolar bone, which is irreversible and results in tooth support loss, as well as the formation of pockets.[1]

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ABSTRACT

Periodontitis is caused by a complex inflammatory over-response, which may be exacerbated by genetic predisposition and environmental factors. Periodontal disease is characterized by the formation of periodontal pockets and the resorption of alveolar bone. The level of bone mass is determined by the balance of bone resorption by osteoclasts and bone formation by osteoblasts. Osteoclasts cause periodontal bone resorption. Receptor activator of nuclear factor-kappa B ligand (RANKL), its receptor activator of nuclear factor-kappa B (RANK), and the decoy receptor osteoprotegerin (OPG) are all key molecules in the regulation of osteoclastogenesis and bone resorption. RANKL/RANK signaling controls the formation of multinucleated osteoclasts from their precursors, as well as their activation and survival during normal bone remodeling. By binding to RANKL and inhibiting its binding to its receptor, RANK, OPG protects the skeleton from excessive bone resorption. This review aimed to investigate variation in the RANKL and OPG levels in the gingival crevicular fluid in periodontal disease.

Keywords: Gingival crevicular fluid, osteoclast, osteoprotegerin, periodontitis, receptor activator of nuclear factor-kappa B ligand, receptor activator of nuclear factor-kappa B.

THE BONE REMODELING PROCESS

Bone is a dynamic hard tissue that undergoes continuous remodeling to meet functional adaptations.[5] The remodeling process is an active and dynamic process that is dependent on the proper balance of bone resorption by osteoclasts and bone deposition by osteoblasts. Osteoclasts are multinucleated cells that destroy the bone matrix, and osteoblasts have osteogenic functions. A proper balance of bone resorption and osteogenic functions is required to maintain a constant bone mass.[6] Due to their mechano-sensorial function, osteocytes, another important cell type derived from osteoblasts, are also involved in the remodeling process.[7] The alveolar bone of the periodontium is not immune to

this process. Alveolar bone remodeling can occur in physiological situations such as occlusal forces, tooth eruption, clinical interventions such as orthodontic tooth movement, and pathological conditions such as periodontitis and periapical pathosis.[8] The RANKL (receptor activator of nuclear factor-kappa B ligand), RANK (receptor activator of nuclear factor-kappa B), OPG (osteoprotegerin) system has been some of the most significant discoveries in bone biology in the last decade. This system is essential for skeletal health, and its disruption leads to or causes a variety of bone diseases.[9] RANKL remodeling, its receptor RANK, and the decoy receptor osteoprotegerin are all key molecules that regulate osteoclast differentiation, recruitment, and function.[10] OPG and RANKL are known to be the primary bone metabolism regulators, as well as being important in the mechanism of periodontal destruction in periodontitis.[11] An increased RANKL/OPG ratio may indicate that the molecular mechanisms of bone resorption are still active and that the corresponding periodontal sites are still at risk of future relapse.

RECEPTOR ACTIVATOR OF NUCLEAR FACTOR-KAPPA B LIGAND

The receptor activator of nuclear factor-kappa B ligand is a type II homotrimeric transmembrane protein that is expressed as both a membrane-bound and a secreted protein. The secreted form is derived from the membrane form either through proteolytic cleavage or alternative splicing. The RANKL gene encodes a protein of 316 amino acids and a molecular mass of 38 kd, the extracellular domains of which self-associate as a trimer. It is also affected by different types of cytokines (IL-1, IL-6, IL-11, TNF-α), glucocorticoids, and parathormone (PTH).[12,13] Osteoblastic lineage cells and activated T cells produce RANKL which promotes osteoclast formation, fusion, differentiation, activation, and survival, resulting in increased bone resorption and bone loss.[6,14] It is mainly expressed in lymph nodes, the thymus, and the lung, and is expressed at low concentrations in a wide range of many other tissues such as the spleen and bone marrow.[15] It is worth noting that three RANKL isoforms have been identified, each of which has the potential to multimerize.[16] RANKL1 has an intracellular, transmembrane, and extracellular domain, whereas RANKL2 has a shorter intracellular domain. RANKL3 is a soluble ligand that lacks both the intracellular and transmembrane domains. These three variants may play different roles or have different potencies in the regulation of osteoclastogenesis, with RANKL1 being the primary inducer and RANKL3 being a potential attenuator.[17]

RECEPTOR ACTIVATOR OF NUCLEAR FACTOR-KAPPA B

The receptor activator of nuclear factor-kappa B is a type I glycoprotein and transmembrane protein from the TNF (tumor necrosis factor) receptor superfamily that is the receptor RANKL. RANK is an essential cytokine for the development and maturation of osteoclast. RANKL stimulates its specific receptor RANK, which is expressed by only a few cell types, including progenitor and mature osteoclasts, activated T cells, and myeloid-derived dendritic cells.[12,18,19] RANK protein expression has been found in the mammary gland[20] and some cancer cells, including breast and prostate cancers, both of which have a high potential for bone metastasis.[4,13,21-23]

OSTEOPROTEGERIN

Osteoprotegerin is a member of the TNF receptor superfamily, with structural homology to RANK.[24] OPG is primarily synthesized by osteoblasts and many organs (lungs, liver, intestines, kidneys, stomach, skin, bones), additionally periodontal tissues, gingival epithelium, and gingival fibroblasts.[25,26] OPG was discovered to be a secreted molecule that inhibits osteoclast differentiation and activity. Osteoblasts secrete an osteoclastogenesis inhibitory factor or decoy receptor; OPG, that specifically binds to RANKL and inhibits RANK-RANKL interaction.[27,28] Because OPG acts as a soluble inhibitor that prevents RANKL interaction and subsequent stimulation with its receptor, RANK, its biological effects are opposed to those of RANKL.[29] Systemic and local stimuli, such as hormones, inflammatory mediators, and bacterial products, regulate the production of RANKL and OPG by various cell types.[30] In the absence of OPG in experiments in mice, osteoporosis was observed with decreased bone density and volume and fractures and deformities.[31,32] This osteoporosis has been found to be reversed with an injection of intravenous OPG. OPG genetically modified mice had osteopetrosis characterized by inhibition of osteoclastogenesis.[24] This data shows that the presence of OPG is necessary for the preservation of bone mass. In RANKL as opposed to OPG, advanced level osteoporosis has been observed in genetically modified mice.[6] In mice without RANKL, osteoclasts completely disappeared and osteopetrosis has been seen to improve.[14,20] According to this data, OPG

is a strong protective agent for bone, while RANKL is a pre-resorptive factor. In addition to its role in bone loss prevention, OPG has been linked to the progression of chronic diseases such as rheumatoid arthritis,[33] cardiovascular disease,[34] and type 2 diabetes complications.[35] These molecular markers have been examined in the context of periodontitis.

RANKL AND OPG EXPRESSION IN PERIODONTAL DISEASE

The tumor necrosis factor superfamily members who have recently been discovered RANKL and osteoprotegerin are important paracrine mediators of bone metabolism and immune functions, and they have been linked to a variety of skeletal and immune disorders and diseases that occur at the interface of bone metabolism and the immune system, such as rheumatoid arthritis. The RANKL/OPG ratio is thought to be the most significant aspect of bone metabolism. In several cases, both an increase in RANKL and a decrease in OPG levels, which act as pro-resorptive signals, stimulate bone absorption.[36] Periodontitis is caused by inflammatory cell infiltration in chronic periodontal disease, which results in bone atrophy. This is reflected in the inflammatory granulation tissue adjacent to the atrophied bone, which has increased RANKL and decreased OPG.[37,38] RANKL to the RANK on the surface of osteoclast precursor cells activates a cascade mechanism inside a maturing bone resorption cell, resulting in the formation of a fully active osteoclast. OPG, a protein from the TNF receptor family, is involved in this process. OPG inhibits osteoclastogenesis by preventing osteoblasts and bone marrow stromal cells from contacting both precursors and mature multinucleated osteoclasts with a RANK receptor. Various studies have found inflammatory cytokines such as IL-1, IL-6, and TNF-α, as well as many other inflammatory cytokines, are important in periodontal disease because they regulate RANKL and OPG expression.[37-39] Previous research found that patients with periodontitis had higher RANKL levels than healthy subjects in gingival crevicular fluid.[40] On the contrary, subjects with periodontitis had lower OPG levels than healthy subjects.[11,40] Mogi et al.[35] recorded RANKL and OPG concentration levels in GCF (gingival crevicular fluid) from periodontitis patients and healthy controls. The group with periodontitis had higher RANKL levels and lower OPG levels. Moreover, when compared to the healthy group, the ratio of RANKL concentration to OPG concentration in GCF was significantly higher in that group.[41,42] These findings suggest that the RANKL/OPG ratio is higher in areas with periodontal activity. Due to changes in RANKL and OPG determining the occurrence of the disease and the level of response to treatment, assessing the RANKL/OPG ratio is more valuable than evaluating OPG or RANKL alone.

RANKL AND OPG LEVELS IN SALIVA

The role of the RANKL-OPG system in periodontal disease is thus well established, as evidenced by an increased RANKL/OPG ratio, which may be a good indicator of molecular diagnostic value for the disease. All current studies have found that RANKL and OPG can be easily identified in gingival tissues and biological fluids such as saliva, serum, GCF. Several studies have also been conducted to determine the presence and levels of RANKL and OPG from whole unstimulated saliva in analysis to define their potential use as salivary biomarkers of periodontal disease.[5,43,44] In one of the initial studies, salivary RANKL concentrations were below detection limits (0.375 pmol/l) in 81% of subjects, regardless of whether they were healthy or CP (chronic periodontitis), whereas TNF-a levels were significantly higher in CP subjects (4.33 pg/mL) compared to healthy subjects (2.03 pg/ml).[43] Other studies in saliva found RANKL concentrations at a range of 20-200 pg/mL[3] or 60-110 pg/mL,[44] and OPG concentrations at a range of 40-250 pg/mL.[5] Nonetheless, the existing literature is insufficient to draw firm conclusions about the diagnostic value of RANKL and OPG in saliva.

EFFECT OF PERIODONTAL TREATMENT ON THE RANKL-OPG SYSTEM

Immunohistochemical findings showed that periodontitis-affected tissue had significantly higher RANKL and lower osteoprotegerin staining than healthy gingival tissue.[11] A high RANKL/OPG ratio is a sign of periodontitis. In an earlier study, the effect of periodontal treatment on the RANKL/OPG system in the GCF was investigated. Significant differences were found in the RANKL/OPG ratio between the healthy and pathologic locations of the study group before periodontal treatment ($p<0.001$), with higher ratios observed in the pathologic locations. According to findings, healthy patients had higher OPG values than treated or untreated patients in the study group. In terms of RANKL levels, higher values were found in the study group's untreated location

with the periodontal condition than in the other locations in both the study and control groups. Furthermore, with the exception of the untreated affected locations, all locations had similar RANKL levels, with no significant differences between them.[42] The GCF analysis after initial periodontal treatment also revealed no changes in RANKL but a decrease in OPG with a potential increase in the RANKL/OPG ratio after four weeks.[5] Despite the improved clinical outcome, a recent study of CP and AP (aggressive periodontitis) patients found that initial periodontal treatment had no effect on the RANKL/OPG ratio in either group after four months of monitoring.[41] In summary, the RANKL levels are higher in areas where there is destructive periodontal activity, which leads to increased osteoclast activity[38,42,45] and RANKL/OPG levels are low in the control group, treated healthy locations, and treated pathologic locations of periodontitis. Contrary to this, the RANKL/OPG ratio was high in locations with untreated periodontitis, which was primarily determined by the high RANKL values.[42] Despite its potential as a biomarker for untreated periodontitis, the RANKL/OPG ratio may not be an accurate predictor of clinically successful treatment results.

In conclusion, periodontal disease is characterized by inflammation and bone loss. Evidence suggests that periodontitis is caused by bacterial factors and antigens that cause a local inflammatory response and activation of the innate immune system. Proinflammatory molecules and cytokine networks are critical components of this process. RANKL and OPG gene expression and tissue localization studies provided concrete evidence that RANKL and OPG are involved in periodontal health and disease. RANK is a transmembrane protein that is expressed in both mature and progenitors of osteoclasts, and its binding to its ligand (RANKL) determines osteoclast differentiation and activation. The osteoprotegerin ligand (OPG-L) is a soluble protein that acts as a RANKL decoy receptor. As a result, OPG acts as an inhibitor of osteoclast formation. This system is essential for skeletal health, and its disruption leads to or causes a variety of bone diseases. The alveolar bone of the periodontium is not exempt from this process. Research indicates that both RANKL and OPG can be detected in the gingival tissue and biological fluids such as gingival crevicular fluid, saliva, and serum. All available studies show that RANKL is increased while OPG is decreased in periodontitis compared to health, or gingivitis. These changes result in an increase in the RANKL/OPG ratio. The RANKL/OPG ratio may be useful as a biomarker for untreated periodontitis or to indicate a prior history of the disease at the site level, but it may not be a reliable indicator of clinically successful treatment outcome.

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