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RANKL, OPG, and BALP Levels in Gingival Crevicular Fluids as a Biomarker on Alveolar Bone Remodeling in the Retention Phase of Orthodontic Treatment

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ABSTRACT

Orthodontic treatment aims to correct malocclusion by applying orthodontic equipment that can transmit mechanical forces to the teeth, resulting in orthodontic tooth movement (OTM). The main problem in this treatment is the occurrence of relapse, so the post-treatment retention phase is critical to prevent relapse. Measuring potential biomarkers of alveolar bone remodeling is a proposed innovation in identifying alveolar bone deposition and resorption in the retention phase. This review highlights the role of essential biomarkers in alveolar bone formation, namely levels of bone alkaline phosphatase (BALP) and osteoprotegerin (OPG) and alveolar bone resorption, namely receptor activator of nuclear factor kappa-B ligand (RANKL) through gingival crevicular fluids. This level measurement is non-invasive and can predict the rate of alveolar bone remodeling to help determine the success of orthodontic treatment.

Keywords: Alveolar bone remodeling, Biomarker, Orthodontic tooth movement, Retention phase

INTRODUCTION

Malocclusion or abnormal dental occlusion is the third priority dental and oral health problem according to the World Health Organization [1]. Untreated malocclusion can cause various issues, including susceptibility to dental caries, periodontal disease, alveolar bone damage, temporomandibular joint abnormalities, and changes in craniofacial development [2]. Orthodontic treatment purposes to place the normal position and occlusion of teeth, improve chewing function, speech, aesthetics, and self-confidence, and prevent dental caries and periodontal disease. Orthodontic treatment applies orthodontic devices that deliver force so the teeth can move [3].

During OTM, the alveolar bone and periodontal ligament (PDL) remodeling occur through the activity of essential cells such as fibroblasts, osteoblasts, osteoclasts and osteocytes. Several key factors are involved in activating osteoclastogenesis, included the RANKL/OPG. RANKL is a essential biomarker secreted by osteoblasts and is responsible for osteoclast recruitment, differentiation, and survival. The binding of RANKL to RANK expressed on the surface of osteoclasts, induces the differentiation of osteoclasts into mature cells. However, OPG, a soluble RANKL receptor generated by

osteoblast, inhibits the differentiation of osteoclast. This negative feedback loop maintains homeostasis between bone deposition and resorption. BALP and runt-related transcription factor (Runx2) are important osteoblast differentiation indicators. BALP is a crucial osteoblast phenotype indicator and essential in bone formation and mineralization [4].

The main problem in orthodontic treatment is the occurrence of relapse, which changes the position of the teeth after orthodontic treatment. The etiological factor of relapse is considered to be multifactorial. Still, the cause that is often proposed is the ongoing process of remodeling of periodontal tissue and alveolar bone around teeth that are moved with orthodontic devices [5,6]. Therefore, evaluation of treatment in the retention phase is critical, where retention is the final phase of orthodontic treatment and functions to maintain the final position of the teeth to prevent relapse.

The biomarkers of alveoar bone remodeling have been studied in the periodontal disease and OTM. The detections of bone biomarkers have been investigated with enzymes and proteins on remodeling phase [7]. RANKL, OPG, and BALP levels in gingival crevicular fluids can be considered as specific measurement of the degree for alveolar bone formation and resorption during retention phase and the assessment of orthodontic treatment success. Herein, we present the review article about the detections of alveolar bone remodeling for the assessment of alveolar bone formation and resorption in the retention phase of orthodontic tooth movement

DISCUSSION

Orthodontic tooth movement (OTM)

The OTM combines the alveolar bone's physiological adaptation to mechanical forces with reversible injury to periodontal tissue through the PDL and alveolar bone remodeling [8]. When orthodontic force is applied to the teeth, the injury will occur to the PDL, forming areas of pressure and tension and triggering remodeling of the PDL and alveolar bone.

The mechanism of alveolar bone remodeling during tooth movement is that pressure on the alveolar bone causes interstitial fluid to be secreted through the canaliculi around the osteocytes and results in tension on the cell surface and extracellular matrix. The fluid flow causes shear stress to the bone extracellular matrix and cell membranes, disrupts integrins, and activates osteocyte signaling cascades. Stress derived from mechanical forces will be transmitted intracellularly from the extracellular matrix via integrins, which induce cascade signals to change gene expression, cytoskeletal organization, proliferation and differentiation, and tissue remodeling [8].

The tension on the osteocyte surface and the extracellular matrix of the alveolar bone and the biological response in the PDL due to the application of orthodontic force synergistically induce osteoclastogenesis followed by alveolar bone osteogenesis. On the stress side, before actual alveolar bone resorption occurs, osteoblasts degrade osteoid through matrix metalloproteinase activity, which aims to make differentiated osteoclasts adhere to the alveolar bone surface. Osteoclasts attached to the bone surface undergo morphological changes and then form a ruffled border for resorption, where the osteoclasts release hydrogen ions to dissolve the inorganic matrix and enzymes such as matrix metalloproteinase and cathepsin to absorb the organic matrix in the alveolar bone [9]. Bone resorption is modulated by RANKL and OPG [10]. On the pressure side, there is an increase in RANKL expression, while in areas of tension, there is an increase in OPG synthesis [11]. RANKL injection was associated with increased osteoclasts and TRAP expression on days 14 and 21 in pressure areas during OTM [12]. Therefore, the RANKL-RANK-OPG system can predict the bone remodelling rate during OTM [11].

On the tension side, osteoblasts are responsible for new bone formation by producing new ECM and then undergoing

mineralization. At the same time, some osteoblasts will be trapped in the bone and turn into osteocytes [13]. Some markers of osteogenesis can also be observed in OTM. Applying mechanical force causes an increase in osterix, which is an osteoblast transcription factor, which will increase alkaline phosphatase activity and mRNA expression of genes specific to osteogenesis such as osteopontin (OPN), bone sialoprotein, osteocalcin (OCN), and collagen. Apart from providing strength, structure and elasticity to bones, type 1 collagen (Col-I) also regulates the expression or secretion of non-collagen proteins such as osteocalcin (OCN), osteopontin (OPN), osteonectin (ONN), and bone sialoprotein (BSP). This shows that the bone formation process involves the coordination of the expression of several molecules, such as growth factors, transcription factors, and anti-inflammatory cytokines [12,14]. Previous research also showed an increase in ALP in gingival fluid on the pulling side of OTM, as it is known that ALP plays a vital role in alveolar bone formation [15].

Retention phase of orthodontic treatment

Orthodontic retention is the final stage of treatment, and the aim is to keep the teeth in their corrected position after completion of OTM. Teeth tend to return to their original position after treatment because the fibres in the PDL are stretched, especially the fibres around the neck of the teeth (interdental and dentogingival fibres) [16,17]. The orthodontist's challenge is establishing conditions in which the PDL and alveolar bone are efficiently remodeled, thus maintaining the new position of the teeth [18].

Over the decades, many theories have been proposed regarding retention. For example, occlusion is critical to stability, and proper function and muscle balance are related to stability. Good orthodontic treatment planning and achieving appropriate occlusal and soft tissue treatment goals can help minimize the occurrence of relapse. Nevertheless, some relapse is almost inevitable unless appropriate retention protocols are implemented after removing active equipment. The results of an experimental study showed significant damage to corrected tooth rotation, lower incisor alignment and overjet in just four weeks when retention appliances were not used after OTM [19]. In addition, the research results on experimental animals showed a relapse of 62.5% of OTM achieved after one day of removal of the orthodontic appliance [20].

Most orthodontic treatment results are potentially unstable; therefore, according to Proffit et al. (2019), retention is needed for three main reasons as follows: 1) gingival and periodontal tissues affected by orthodontic movements require time to reorganize when the orthodontic appliance is removed, 2) the tooth may be unstable after treatment, resulting in constant soft tissue pressure and a tendency to relapse, and 3) changes caused by growth can alter the results of orthodontic treatment [21].

Alveolar bone biomarker on orthodontic tooth movement

Biomarkers are substances measured and evaluated objectively as indicators of normal biological processes, pathological processes, or pharmacological responses to therapeutic interventions. A promising biomarker must be specific and sensitive. It can inform about biological conditions regarding periodontal tissue changes and their relationship to the phases of tooth movement to shorten the treatment period and help avoid the adverse effects of orthodontic treatment [22].

RANKL

The RANKL, which is a homotrimeric protein, is a tumour necrosis factor (TNF) family produced by osteoblasts, osteocytes, activated T cells, and bone marrow stromal cells. Secreted RANKL results from proteolytic cleavage or alternative splicing in the membrane. Matrix metalloproteinase (MMP3 or MMP7) and a disintegrin and metalloprotease domain (ADAM) play a role in the proteolytic cleavage of RANKL.

RANKL secreted by preosteoblasts, osteoblasts, osteocytes, and periosteal cells binds to the RANKL receptor (RANK) expressed by osteoclasts and their precursors to stimulate preosteoclast differentiation, attachment, activation, and osteoclast maturation [23,24].

The RANKL can bind to both OPG and RANK receptors. OPG is a product of osteoblasts and B cells, a member of the 380-amino acid TNF receptor superfamily that functions as a decoy receptor for RANKL. RANKL, when bound to OPG, will inhibit osteoclast differentiation, while bound to RANK will induce osteoclast differentiation. RANKL primarily induces the RANK co-receptor to initiate the recruitment of TNF-receptor associated protein (TRAP), of which TRAF6 plays an important role. TRAF6 acts through an inhibitor of NFκB (IκB), which generally sequesters and inhibits NFκB transcription, and IκB kinase (IKK), which modulates IκB phosphorylation. Once activated, NFκB will encourage transcription of NFATc1 in the cell nucleus. As is known, NFATc1 plays a role as the primary modulator of osteoclastogenesis [25]. RANK signals to GRB2-associated binding protein 2 (GAB2) via Src kinase activate PI3K/Akt signaling that drives NFATc1 expression. NFκB and PI3K/Akt together increase the transcription of other osteoclast differentiation markers, including cathepsin K (CTSK), MMP, and tartrated-resistant acid phosphatase (TRAP) [13].

The RANKL is a ligand-receptor for osteoclast formation, located on or cleaved from the cell membranes of osteoblasts, osteocytes, T cells and B cells. RANKL works mainly through the NFκB pathway (Nakashima et al., 2011). During OTM in RANKL deletion mouse models, it was reported that osteoclast formation was blocked. These results indicate that RANKL produced by PDL and bone lining cells plays a vital role in osteoclastogenesis [26].

OPG

Osteoprotegerin (OPG) is a TNF receptor family member, mainly expressed by bone marrow stromal cells and osteoblasts. OPG functions as a RANKL decoy receptor that does not have a transmembrane domain. OPG can bind RANKL to prevent RANKL from binding to RANK receptors and inhibit osteoclastogenesis [23]. OPG can be measured in serum, plasma EDTA, citrate, and heparin serums. There are commercially available sandwich ELISA tests for analyzing OPG using monoclonal and polyclonal detection antibodies. However, applying serum OPG as a biomarker for evaluating bone marker activity requires additional investigation [7].

The RANKL/OPG ratio can be used as a determinant of bone resorption. Several factors can influence the regulation of the RANKL-RANK-OPG system, including cytokines (TNF-α, IL-1, IL-6, IL-4, IL-11, and IL-17), hormones (vitamin D, estrogen, and glucocorticoids), as well as mesenchymal transcription factors. OPG regulation also involves the Wnt/β-catenin signaling pathway [24]. The RANKL/RANK/OPG system is vital in OTM. The RANK-RANKL-OPG is essential to the differentiation of osteoclast during tooth movement. Previous studies showed that RANKL levels in GCF have elevated, while OPG levels have been suppressed 24 hours after orthodontic force application, suggesting alveolar bone resorption [27].

BALP

The ALP is a glycoprotein and functions as an ectoenzyme that attaches to the outer surface of cells and matrix vesicles. ALP is a large family of dimeric enzymes hydrolyze various monophosphate esters at high optimal pH by releasing inorganic phosphate. There are four genes of ALP isozymes, namely intestinal ALP (IALP), placental ALP, germ cell ALP, and tissue-nonspecific ALP (TNALP), which are expressed by bone (BALP), liver, and kidney [28,29]. BALP and TRAP, which are phosphatase enzymes, are essential bone mineralization regulations but have different roles. BALP plays a role in the mineralization process originating from osteoblasts, while TRAP plays a role in

osteocyte lacunae and areas of bone resorption by osteoclasts [29].

The BALP is an essential marker of bone formation. BALP can be used to determine the influence of pharmacological and mechanical stimuli on cortical bone even though the bone resorption process is irreversible. In the OTM model with RANKL injection, BALP expression was higher in the tension area compared to the pressure area. However, on day 21, BALP expression showed an increase in stress areas. The results of this study are by the theory of tooth movement, where in the initial period of tooth movement, there is an increase in bone formation in the tension area followed by an increase in bone formation in the pressure area in the resorption area [12].

Examination of RANKL, OPG, and BALP levels in gingival crevicular fluids

The GCF is an essential biomarker in orthodontic treatment screenings. Its measurement can quickly identify the alveolar bone resorption and deposition on the tension and pressure side. The GCF can also help predict the alveolar bone remodeling in the retention phase of OTM. However, the application of this method in clinical still needs to be improved, with further studies of potential GCF markers for orthodontics [30].

The quantifications of GCF and its constituents are a contemporary method of identifying potential biomarkers with reasonable accuracy. In orthodontics, biomarkers related to bone formation (BALP and OPG) show new strategies for alveolar bone regeneration [13]. Alveolar bone resorption biomarkers (RANKL) can also be detected in GCF [31] and can predict relapse prevention. Most studies used paper points in experimental animals and paper strips in human samples. These paper points were considered more efficient in GCF collection because they could be inserted easily into the gingival sulcus and absorb fluids in it for 30-60 seconds [31].

CONCLUSION

Biochemical markers of alveolar bone remodeling in the retention phase of OTM have contributed to a better understanding of bone resorption and deposition during this phase. Although not considered appropriate for assessing orthodontic treatment success, they can provide additional and complementary information on the degree of alveolar bone remodeling. Currently, the most sensitive markers of alveolar bone formation are OPG and BALP, whereas the resorption markers are RANKL. However, further research and studies are still needed to assess the effectiveness of measuring these biomarkers in orthodontic treatment.

Declaration by Authors

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