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Review

Orthodontics

Biomarkers of periodontal tissue in gingival crevicular fluid during orthodontic movements: An overview

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Abstract

Introduction

Only in recent times has the importance of gingival crevicular fluid in periodontal health and in particular in maintaining the integrity of periodontium during application of orthodontic forces been enhanced. The aim of this short review is to evaluate the importance of substances as valid biomarkers of periodontal health during orthodontic movements.

Conclusion

The conclusion is that GCF is a powerful vehicle for clinical diagnostics, since it contains different biochemical and cellular arrays in relation to different clinical situations indicative of the state of periodontal health during orthodontic treatment.

Introduction

Gengival crevicular fluid (GCF) is an important source of biomarkers related to the state of health of deeper-seated tissues of periodontium. These properties can be particularly useful in monitoring the effectiveness of orthodontic treatment and the response of the alveolar bone to orthodontic forces.

In recent years, thanks to sophisticated biochemical analysis, several biomarkers of alveolar bone have been discovered. These biomarkers change in special clinical situations, such as severe periodontal disease, osseointegration, trauma due to orthodontic movements and are therefore indicative both of active pathology or healing. This review aims to assess the current knowledge about biomarkers of periodontal tissues during orthodontic treatment.

In fact, the clinical parameters do not allow to assess the state of suffering of the periodontium, especially alveolar bone remodelling during orthodontic treatment. Gengival crevicular fluid allows very specific and non-invasive biochemical analysis, so the study of biomarkers of GCF is a very exciting prospect for future clinical applications.

The biological mechanism that controls the passage from the stimulus, application of continuous orthodontic forces, to the reaction, displacement of the tooth in the periodontal space, can be evaluated in accordance with the theory of tension-pressure and cell differentiation, resulting in tooth movement or retention controlled by biochemical signals[1,2].

Recent reviews describe the main gingival crevicular fluid (GCF) biomarkers related to orthodontic movements and classified them into four main groups: biomarkers of inflammation, bone metabolism, cell death and bone deposition and mineralization.

Biomarkers of inflammation: Interleukins (IL-1β, IL-6, IL-8), Tumour Necrosis factors (TNF-α), Colony-stimulating factors (M-CSF, G-CSF, GM-CSF), Prostaglandins (PGE), Vascular endothelial growth factors (VEGF), Calcitonin gene related peptide (CGRP), Substance P.

Interleukins (IL-1 β, IL-6, IL-8) are cytokines involved in the bone remodelling, bone resorption and neo-bone apposition during orthodontic movements[3,4,5,6].

IL-1 β s the most potent cytokine stimulating osteoclast activity and attracting white blood cells and other cellular mediators in the process of bone remodelling. It is the first polypeptide regulating the processes of resorption and neo-bone apposition in relation to mechanical stress.

Moreover, IL-1 β is one of the mediators of inflammation which induces the secretion of substances causing pain. Besides, IL-1 β is produced by the periodontal ligament in a quantity sufficient to diffuse into the gingival crevicular fluid and has been identified as a biomarker of orthodontic movement.

Interleukin-6 (IL-6) is a cytokine involved in the process of bone resorption. IL-6 promotes the differentation of osteoclast cells and can activate mature osteoclasts[7,8].

Moreover, IL-6 is involved in osteoclastogenesis through stromal or osteoblastic cells[9], infact the level of IL-6 in gingival crevicular fluid (GCF) increases during orthodontic tooth movement[10]. These findings suggest that IL-6 may play important roles in bone resorption during orthodontic tooth movement.

Several studies have demonstrated increased level of IL-8 at periodontal deep ligament (PDL) tension sites, so IL-8 is a promoting factor for bone remodeling[11]. Increased levels of these proinflammatory cytokines are demonstrated in GCF during orthodontic tooth movement.

Tumour Necrosis factors (TNF-α) is another potent proinflammatory cytokine that stimulates acute or chronic inflammation and bone resorption, favouring the production of osteoclasts in the presence of Colony-stimulating factors (M-CSF, G-CSF, GM-CSF)[12].

Macrophages-Colony-Stimulating Factors (M-CSF) are glycoproteins promoting the activity of monocytes-macrophages and granulocytes. In mice they interact in bone remodelling during the movement of the teeth[13]. The M-CSF are produced in the early phase of bone remodelling, stimulating osteoclasts maturation[13].

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Competing interests: None declared. Conflict of interests: None declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

FOR CITATION PURPOSES: *Lauritano et al. Biomarkers of periodontal tissue in gingival crevicular fluid during orthodontic movements: An overview. OA Dentistry 2014 Jan 18;2(1):1.*

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Prostaglandins, produced by deformed osteoblasts and gingival fibroblasts, are cytokines implicated in inflammation provoked by orthodontic tooth movement. Among the subclasses of prostaglandins, prostaglandin E2 (PGE2) is strongly related to bone resorption[14].

Prostaglandin E2 (PGE2) is induced by interleukin-1 β. IL-1 β synergically upregulate the formation of prostaglandins in the periodontal tissue subjected to stress orthodontic. Orthodontic and orthopaedic forces evoke changes in the levels of inflammatory mediators in the periodontal tissues and can trigger the processes of bone resorption in tissue around the tooth root[15].

Vascular endothelial growth factor (VEGF) is a cytokine involved in tissue neoformation since it increases vascular permeability and promotes angiogenensis.

During orthodontic movements compressive forces induce the formation of new capillaries by the activation of VEGF[16,17].

During orthodontic therapy the increase of concentration of biologically active proteins contribute to increase neurogenic inflammation. Nociceptive somatosensory neurons transmit signals from the peripheral fibres of the periodontal tissues to the central nervous system.

With the application of orthodontic forces the peripheral nervous system periodontium fibres release calcitonin gene related peptide (CGRP) and substance P also acting as vasodilators, increasing vascular flow and permeability (diapedesis) and stimulating plasma extravasation and leukocyte migration into tissues (transmigration). Furthermore, the substance P promotes the secretion of proinflammatory cytokines by the immunocompetent system and stimulates the production of PGE2[18].

Biomarkers of bone metabolism: Osteoprogerin (OPG), Receptor activator of nuclear factor kappa-B (RANK), Receptor activator of nuclear factor kappa-B ligand (RANKL).

Osteoprotegerin (OPG), receptor activator of nuclear factor-(KB) ligand (RANKL), and its decoy receptor RANK, are protein ligands. They share similarities to the superfamily of receptors for tumour necrosis factors and function as paracrine regulators of bone metabolism and osteo-clastogenesis. Osteoprotegerin lacks transmembrane and cytoplasmic domains and is secreted as a soluble protein, mainly by osteoblastic lineage cells. The primary biologic actions of OPG are inhibition of osteoclast differentiation, inhibition of osteoclast resorptive function, and stimulation of osteoclast apoptosis[19].

RANK is a peptide seated on the cell surface of osteoclast precursors. RANKL is another peptide induced by osteoblastic lineage cells and activated T-cells. RANK expressed by the cell line is cell-bound, otherwise is soluble RANK (RANKLs) if expressed by T-lymphocytes Promotion, fusion, differentiation, activation, survival of osteoclasts in promotion by RANKL, together with another very important protein ligand, M-CSF, thus inducing bone resorption. RANKL binds to RANK and expresses its biological effects. OPG acts as a soluble receptor antagonist that neutralizes RANKL preventing RANKL-RANK interaction, the biological effects of OPG are opposite to the RANKL-mediated effects[20].

These ligands appear to be the key regulators in the process of bone remodelling. During orthodontic tooth movement, on the compressed side of the tooth, RANKL expression is induced. On the contrary, on the tensile side of the tooth, there is an increase in OPG synthesis. The relative expressions of OPG and RANKL on the tensile and compressed sides of the tooth during orthodontic tooth movement regulate bone remodelling.

The process of osteoclastic bone resorption is similar to those of root resorption. Infact, the alveolar remodelling is functional to the OPG/RANKL/RANK coordination and is quite similar to the physiological root resorption induced by orthodontic tooth movement. During physiological root resorption, in the dental follicle environment, the ratio of OPG to RANKL supports, rather than inhibits, osteoclastinogenesis. The dental follicle and/or the stellate reticulum releases cytotrophic factors, such as parathyroid hormone-related peptide (PTHrP), interleukin-1 β, and transforming growth factor-α1, inducing the expression of RANKL during permanent tooth eruption. During orthodontic tooth movement, this RANKL to OPG ratio in periodontal ligament cells also contributes to root resorption. A huge amount of RANK is produced in case of severe external apical root resorption by the compressed periodontal ligament cells and this process up-regulates osteoclastogenesis. This explains the greater increase of RANKL and decrease of OPG in cases of severe root resorption[20].

RANKL and OPG are important factors for the regulation of bone remodelling as well as root resorption in periodontal tissues. The regulation of bone homeostasis by the OPG/RANKL/RANK system and their concentrations are determined by serum OPG and sRANKL levels, and they might be useful biomarkers for predicting the rate of bone remodelling during orthodontic tooth movement.

OPG and sRANKL are produced from several sources and their concentrations may be influenced by different physiological and pathological processes, so it would be of great interest to investigate whether serum and gingival crevicular fluid (GCF) concentrations of RANKL and OPG could be related to the degree of root resorption induced by orthodontic therapy[20]. Biomarkers of cell death: Caspase-1, B-glucuronidase (βG), Aspartate aminotransferase (AST), Lactate dehydrogenase (LDH).

The capsasi-1 is the main mediator of the apoptotic response triggered by changes in the intracellular fluid. The capsasi-1 has the function to process and activate the pro-inflammatory cytokine interleukin proIL-1β and other proinflammatory interleukins[21].

The main biomarkers implicated in the release of polymorphonuclear leukocytes is the lysosomal enzyme β-glucuronidase (βG). Increased levels of this enzyme were observed in the GCF of adolescents undergoing orthodontic treatment with rapid palatal

Competing interests: None declared. Conflict of interests: None declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
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expander[22]. However, the β-glucuronidase is released during application of direct and indirect mechanical forces to the teeth.

During orthodontic movement in periodontal sites undergoing compression other enzymes are measured such as the aspartate aminotransferase (AST) and lactate dehydrogenasi (LDH). These enzymes usually present in cell cytoplasm, are released into the extracellular environment after cell necrosis[23,24].

Biomarkers of bone deposition and mineralization: Osteoprotegerin (OPG), Bone alkaline phosphatase (ALP). Osteoprotegerin (OPG) acts on bone cells in terminal stages of osteoclast differentiation, suppression of activation matrix of osteoclasts, and induction of osteoclast apoptosis[19].

Bone metabolism is linked with alkaline phosphatase (ALP) and acid phosphatase (ACP), expressed, respectively, by osteoblasts and osteoclasts. During orthodontic therapies these enzymes diffuse into GCF from the periodontium. This process has been demonstrated in human and animal models[25]. Enzyme alkaline phosphatase (ALP) is commonly associated with bone metabolism, infact osteoblasts show high alkaline phosphatase concentration[26].

Moreover in bone pathologies such as osteitis deformans, associated with osteoblastic activity, ALP levels are increased by 10-25-folds[26]. Transient increase in activity is also observed during healing of bone fractures and physiologic bone growth[26]. In fact injured, damaged or dead cells release acid and alkaline phosphatases into extracellular tissue fluid, moreover as a result of orthodontic force application, these enzymes, produced in the periodontium, diffuse into the GCF[26]. Aspartate aminotransferase (AST) is a soluble enzyme released into the extracellular environment upon cell death, when it is normally confined to the cytoplasm of cells[27].

The presence of AST enzyme has been demonstrated in GCF and several studies have observed that AST activity in GCF are related to the amount of periodontal tissue destruction in periodontitis[27].

Therefore, it has been suggested that AST levels in GCF may represent a potential marker for monitoring the periodontal metabolism. These enzymes are reported to be good indicators of bone metabolism and periodontal tissue remodelling associated with orthodontic tooth movement.

Discussion

GCF is a powerful vehicle for clinical diagnostics, since it contains different biochemical and cellular arrays in relation to different clinical situations indicative of the state of periodontal health during orthodontic treatment[28].

GCF can be considered as an exudate or transudate. It's a fluid arising from the gingival margin, that can be collected in a non-invasive and site-specific way. The results of several studies suggest that GCF is the product of vascular tissue in the gums, where the effect of trauma on the capillaries produces the gingival fluid. In periodontal disease there is an evident increase of GCF due to the loss of continuity of the basement membrane of the junctional epithelium.

This process is accompanied by a widening of the intercellular spaces of the junctional epithelium and by a partial destruction of the basal membrane. These events lead to an increase of the osmotic gradient for the formation of a half-waterproof membrane. The different osmotic gradients drain fluid from the capillaries of the periodontal tissue.

The initial exudate is usually serous, but later is contaminated by inflammatory molecules making it an exudate containing biomarkers that represent the metabolic state of deep periodontal tissues. The importance of GCF in the assessment of orthodontic movements could be influenced by different parameters that do not typically relate to inflammation and bacterial plaque. The trauma of the alveolar bone resorption at the level of the deep periodontal apparatus induces a pressure on the surrounding tissues which determines an increase of production of GCF, which can be used to test the factors influencing orthodontic movements[29]. This short review has focused attention on current knowledge about biomarkers of GCF in relation to orthodontic movements.

Conclusion

The definition of a package of biomarkers could help us in making a better diagnosis and treatments more precise, thereby providing orthodontists additional information that cannot be deduced from clinical parameters.

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Competing interests: None declared. Conflict of interests: None declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
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Competing interests: None declared. Conflict of interests: None declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
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