



Review Article

Biomarkers in orthodontics: A review

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ABSTRACT

The examination of Gingival crevicular fluid (GCF) may be considered an acceptable way to depict the biochemical changes occurring during orthodontic tooth movement. Correlating the changes taking place in GCF with different types of orthodontic forces, the patient can be managed based on individual patients' tissue response. Thus, this can be an effective way of improving treatment efficiency and results. There is little evidence regarding which GCF biomarkers are associated with the growing phase. Most of the earlier reports provide information about correlation of GCF biomarkers with inflammation, bone remodeling and tissue damage and other processes associated with orthodontic tooth movement. This method is not being clinically used to its full diagnostic potential and requires further studies to provide additional data.

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1. Introduction

Orthodontic tooth movement occurs due to complex interactions and interplay between alveolar bone, periodontal ligament (PDL) cells. The inflammatory changes produced by orthodontic forces lead to tooth movement and remodeling changes.¹ An expression of this phenomenon is found in the GCF of the teeth being moved. There is an increase in concentration of neurotransmitters, growth factors, cytokines etc. Orthodontic forces disrupt the homeostasis of the extracellular matrix of PDL and alters composition of GCF. GCF can be labelled as a transudate or an exudate.² Analysing the biomarkers, allows for a better understanding of the PDL changes associated with movement of teeth. The vascular changes and leucocyte infiltration bring about the remodeling of alveolar bone during orthodontic tooth movement.^{3,4} These changes depend upon the amount, direction and duration of force applied.⁵

1.1. Biomarkers

A biomarker is a substance that can be measured and evaluated to depict or indicate normal biologic, pathogenic or pharmacologic response to a therapeutic intervention.⁶

High specificity and sensitivity are 2 main characteristics that should be associated with a good biomarker. The treatment duration can be shortened by acquiring knowledge about the type of cellular process, which, in turn will help in using optimum force levels.²

1.2. Metabolic products of paradental remodeling

1.2.1. Markers of orthodontic tooth movement

1.2.1.1. Glycosaminoglycans. Extracellular matrix of connective tissues contains Glycosaminoglycans (GAGs).⁷ The GCF volume increases and reduces during retention due to changes in gingival inflammation during orthodontic tooth movement. The GAGs, chondroitin sulphate levels change during retention.⁸ During orthodontic treatment, the levels of chondroitin sulphate change in the deeper periodontal tissues and PDL. Samuels et al showed that the levels of GAG varied depending on the type of tooth movement. Monitoring the levels of Chondroitin sulphate

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during orthodontic tooth movement is helpful in obtaining optimum treatment results.⁹

Table 1: Phases of orthodontic tooth movement (Pilon et al)

Phase 1 Initial	24H-2Days Initial tooth movement within the socket.	Acute inflammatory response Vasodilation-migration of leucocytes-release of cytokines-cell signalling molecules (metabolic products of paradental remodeling)
Phase 2 Arrest	20-30 days Movement stops	Chronic inflammation Continuation of migration of leucocytes Paradental remodeling
Phase 3 Acceleration	40 days of accelerated tooth movement after initial force application	Another period of acute inflammation superimposing the on-going chronic inflammation
Phase 4 Linear	Overall tooth movement	Recruitment of macrophages, fibroblasts, osteoblasts, and osteoclasts. Alkaline phosphatase activity

1.3. Osteocalcin

Osteocalcin (OC) is an important component in the extracellular matrix of bone.¹⁰ It is a specific biomarker produced by osteoblasts and correlates with active osteoblastic activity.¹¹ Structurally, osteocalcin binds to collagen and apatite present in bone and helps in remodeling of bone. The GCF of patients with periodontal disease presents with osteocalcin and an increase in its concentration has been shown to be associated with higher rates of bone turnover.^{12,13}

1.4. Matrix metalloproteinases 1 and 8

In pathological and physiological conditions, the PDL remodeling is mainly controlled by the enzyme Matrix metalloproteinases (MMPs). Osteoclasts cause bone resorption by demineralization of the inorganic portion of bone by acid and degradation of organic component of bone by cathepsin K and MMPs.^{14,15} Collagenase-1 (MMP-1) and collagenase-2 (MMP-8) are matrix metalloproteinases that initiate tissue remodeling by cleaving native triple-helical interstitial collagen. An experiment done on dogs demonstrated an increase in MMP-1 levels during orthodontic force application, which later decreased after force removal.¹⁶ However, there was inconclusive evidence of MMP-1 in GCF of patients undergoing orthodontic treatment.¹⁷

1.5. Inflammatory mediators

1.5.1. Prostaglandin E

Prostaglandin E2 (PGE2) is a compound that is derived from arachidonic acid and acts along with proinflammatory hormones as a potent biochemical mediator of inflammation. The level of Prostaglandin E2 increases during orthodontic tooth movement and is a marker of bone resorption. Application of Orthodontic force stimulates the cells in the local environment to synthesize and secrete Prostaglandin E2, which, in turn, will bring about osteoclastic bone resorption.¹⁸ Interleukin-1 controls the production of Prostaglandin E2. Prostaglandin E2 levels in Gingival crevicular fluid were found to be highest 1 day after force application and reduced to normal levels within 7 days.^{19,20}

1.5.2. Neuropeptides (calcitonin related gene peptide and substance p)

According to Rosenfeld et al. substance P and CRGP coexisted in the sensory ganglion neurons having a small to medium diameter. In cats, there was an increase in nasal blood flow which was concentration dependant when substance P or CRGP was infused in the local arteries (Stjarne et al, 1989). According to Wakisaka, in cat's pulp, the sub-odontoblastic zone showed CRGP like immunoreactivity in nerves along blood vessels. The dental pulp in cats underwent vasodilation which was 10 times more when CRGP was administered after substance P than before it (Gazelius et al, 1987). Intensified CRGP immunoreactivity was seen 5 days after commencement of molar movement and was present mainly in the tension site. In cats, following orthodontic force application to the maxillary canines at intervals of 1hr, 2 days, 7 days, 28 days, a similar pattern of increase in cellular staining for CRGP was observed (Okamoto et al, 1991). Neurotransmitters such as Substance P, VIP and CRGP may play a dual role in the mechanically stressed periodontium. One being, its action on the endothelial cells thereby promoting vasodilation and facilitating diapedesis, and the other being, the regulation of neuropeptides and its activity after introducing them to specific receptors following release from sensory nerve endings.

1.6. Interleukin-1 (receptor antagonist) 1 β ,2,6,8

Many cell types, such as fibroblasts, osteoclasts and polymorphonuclear leukocytes (PMNs) release proinflammatory cytokines, one of which is Interleukins (ILs). The production of Interleukins is time dependant and are released during periodontal remodeling process following orthodontic force application.²¹ Interleukins are used as biomarkers to understand the metabolic processes associated with orthodontic tooth movement because, they play a role in normal physiologic turnover

of bone and remodeling process following application of mechanical stress.^{2–25} The Interleukins 1,6 and 8 are proinflammatory interleukins, that can be found in the GCF during orthodontic tooth movement.²⁶ Interleukin-1, a proinflammatory cytokine that is produced by activated macrophages, monocytes, B-cells, neutrophils, fibroblasts, and epithelial cells, is a potent stimulator of bone resorption. The interleukins play a role in the proinflammatory process, wound healing and matrix degradation.²⁷ In response to inflammation, many cells such as fibroblasts, epithelial cells, endothelial cells and alveolar macrophages, produce and secrete Interleukin-8. The Interleukin-8 is a potent proinflammatory cytokine which helps in recruitment and activation of neutrophils throughout inflammation. Therefore, inflammatory cells like neutrophils migrate from the PDL capillaries to the inflammatory region. Interleukin-6 is a macrophage which originates from the T-cells. The accumulation of Interleukin-6 in the connective tissue adjacent to the periodontal pockets affects healing of the periodontal pockets as a result of increased synthesis or reduced release into the GCF.²⁸ Study reports have shown that Interleukin-1b can stimulate bone resorption during orthodontic tooth movement.^{29–31}

1.7. Tumour necrosis factor-alpha

Tumour necrosis factor-a (TNF-a) is a proinflammatory cytokine that is derived from monocyte/macrophage and stimulates synthesis of proteolytic enzyme and osteoclastic activity. It is another proinflammatory cytokine that is involved in bone resorption, acute and chronic inflammation and has been investigated in orthodontic tooth movement. Activated monocytes, macrophages, osteoblasts, epithelial cells and endothelial cells produce tumour necrosis factor-alpha.³² Tumour necrosis factor-alpha is also an apoptotic factor for osteocytes. This could function as a signal for osteoclast recruitment for bone resorption in the side undergoing PDL pressure. This also simultaneously inhibits osteoblasts that are linked covalently in the native state to a core protein to form proteoglycans.³³

1.8. Receptor activator of nuclear factor-kappa/receptor activator of nuclear factor-kappa ligand/osteoprotegerin system

The salivary RANKL and OPG level seemed to correspond with its levels in the GCF. This hypothesized the fact that salivary RANKL and OPG were derived from the GCF. The level of RANKL increased after each activation appointment and corresponds to the time when active tooth movement is taking place. The OPG levels reduced during active tooth movement. Therefore, the RANKL/OPG ratio increased post the activation appointment during orthodontic tooth movement.^{34,35}

1.9. Enzymes of high cellular activity

1.9.1. β -Glucuronidase

b-glucuronidase is a lysosomal enzyme that is elevated during degradation process of connective tissue and is associated with release of primary granules from the neutrophils. A significant increase in b-glucuronidase levels is seen 2 weeks after activation of orthodontic appliance.³⁶

1.9.2. Aspartate aminotransferase and lactate dehydrogenase

During apoptosis, Aspartate aminotransferase (AST) is released from the cytoplasm into the extracellular environment. The amount of tissue destruction and bone remodeling occurring during orthodontic tooth movement can be assessed by evaluating the increase in levels of AST in the GCF.^{37,38} During apoptosis, Lactate dehydrogenase (LDH) present in the cytoplasm is released extracellularly. There is a Positive correlation existing between LDH levels and orthodontic tooth movement.^{39–41}

1.10. Enzymes and enzyme inhibitors

1.10.1. Cathepsin B

Cathepsin B (CAB) is a multifunctional biomarker and an intracellular lysosomal enzyme that initiates and maintains the inflammatory process. It is also associated with degradation of extracellular components like collagen. The levels of CAB are high, 1 day after starting orthodontic treatment and corresponds to the inflammatory process occurring during tooth movement.⁴² The CAB levels are high even 1 month post orthodontic treatment due to the collagen degradation and decomposing of exposed collagen fibers.⁴³

1.10.2. Acid phosphatase and alkaline phosphatase

Bone turnover can be assessed by monitoring acid and alkaline phosphatase (ALP) activity in tissues. Bone resorption leads to an increase in in acid phosphatase activity, whereas, bone formation is associated with an increase in alkaline phosphatase activity.⁴⁴ Typically, alkaline phosphatase levels will be increased during initial stages of tooth movement, and an increase of acid phosphatase will occur in the later stages of tooth movement.⁴⁴

1.11. Role of CCR2

Regulation of bone remodeling during orthodontic tooth movement is carried out by Cytokines and chemokines. During mechanical loading, CC chemokine ligand 2 (CCL2) level is increased and functions to recruit osteoclasts. Absence of CCR2 leads to a reduction of osteoclastic and osteoblastic activity. The CCR2-CCL2 axis is related to osteoclast recruitment, bone resorption, and orthodontic

Table 2: List of GCF biomarkers and their role in orthodontic tooth movement

Inflammatory mediators	
Prostaglandin E-2	Bone resorption
Substance P	Bone resorption
Epidermal growth factor	Bone resorption
Transforming growth factor	Bone remodeling
Rankl	Stimulation of osteoclastic differentiation
Osteoprotegerin	Inhibition of osteoclastic differentiation
Granulocyte macrophage colony stimulating factor	Bone turn over
Alpha-2 microglobulin	Enhancer of IGF-1
Interleukin 1 β ,2,6,8	Bone remodeling
Myeloperoxidase-enzyme in PMN	Inflammation
Metabolic products of paradental remodeling	
Hyaluronic acid	Indicator of breakdown of gingival tissue
Chondroitin sulphate	Indicator of breakdown of alveolar bone and PDL
Pentaxrin-3	Marker of inflammation
Osteocalcin	Bone turnover
Insulin growth factor	Regulators of cell differentiation and apoptosis
Pyridinoline,deoxy pyridinoline	Indicators of bone metabolism
N-telopeptide	Bone resorption
Dentin matrix protein	Root resorption
Enzymes	
Acid phosphatase	Bone resorption
Alkaline phosphatase	Bone formation
Aspartate amino transferase	Cell necrosis
Cathepsin B	Extracellular matrix degradation
Matrix metalloproteinases(1,2,8)	Breakdown denatured collagen
B glucuronidase	Marker of granule release by PMN
Lactate dehydrogenase	Indicator of cell death

GCF: Gingival crevicular fluid, IGF-1: insulin like growth factor, GAG: Glycosaminoglycans
 PMN: Polymorphonuclear neutrophils, TNF: Tumour necrosis factor, PDL: Periodontal ligament

tooth movement. Therefore, modulating the extent of orthodontic tooth movement can be done by blocking the CCR2-CCL2 axis.⁴⁵

1.12. Polycystin-1

PC-1 is involved with the remodeling of bone. An experiment was done on mice, wherein, stress was produced across the periodontal ligament to induce bone remodeling. It was found that in PC-1 deficient mice, no molar tooth movement was observed. PC1-deficient mice also exhibit premature ossification of the pre-sphenoid synchondrosis and retarded postnatal growth of the anterior craniofacial

complex.¹⁷ PC-1 deficient mice showed a difference in the osteoclastic activity, which was not reported earlier. This difference in osteoclastic activity may be due to lack of signal from the PDL. This suggests that PC-1 is involved in osteoclast formation.⁴⁶

1.13. Colony stimulating factor

They are glycoproteins which regulate the synthesis, maturation and function of monocytes and granulocytes. Endothelial cells and fibroblasts synthesize M-CSF. Kahn and Simmons demonstrated that osteoclasts can be produced by culturing M-CSF and bone marrow cells for 10 days. According to Takahashi, M-CSF is the most potent in stimulating bone cells to produce osteoclasts.⁴⁷

The above review of signal molecules that modulate various steps of tissue remodeling introduces the orthodontist to the complexity and minute details of events that appear to have major roles in this process. Clinically, orthodontic patients might sense pain shortly after appliance activation. However, this feeling is just one of the many reactions on the cellular and molecular levels that typify orthodontic tissue remodeling.

2. Conclusion

Numerous biomarkers have been reported to be found in the GCF during the course of orthodontic tooth movement. These biomarkers provide vital information about the micro-environment. The biomarkers in the GCF reflect the changes occurring during orthodontic treatment. Knowledge of biomarkers gives information about the proper choice of mechanical loading, which thereby helps in improving patient comfort and reducing treatment time.

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4. Conflict of Interest

The authors declare they have no conflict of interest.

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