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RESEARCH ARTICLE

THE MULTIFACETED CAMEO OF BIO-MARKERS IN ORTHODONTIC TOOTH MOVEMENT – AN OVERVIEW

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ABSTRACT

Orthodontic tooth movement necessitates a considerable amount of periodontal tissue remodelling. During orthodontic therapy, teeth are moved via the processes of osteoclast bone resorption and osteoblastic bone formation. Hormones, cytokines, growth factors and elements of the bone matrix are some of the significant elements that are involved in the process of osteo-clastogenesis. Gingival crevicular fluid (GCF) has been revealed to include the biomarkers responsible for both bone remodelling and alveolar degradation. As a result, research has indicated that GCF may be a good indicator of the immunological and inflammatory responses brought on by both periodontitis and the use of orthodontic treatment. Examination of GCF is a reliable way to show the biochemical alterations that takes place during orthodontic tooth movement. Damage to the periodontal tissues during the course of orthodontic therapy, leads a variety of physiologically active substances which represent bone deposition and breakdown processes. For the current narrative review, a bibliographic search was done in PubMed and other databases for English articles that were published since last decade. Clinically relevant knowledge of the biomarkers of GCF enables better mechanical force selection for better orthodontic therapy with fewer side effects and shorter treatment periods. The main aim of the present narrative review is to provide a better understanding of the underlying bio-mechanisms of orthodontic tooth movement which are accomplished by various markers.

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INTRODUCTION

Orthodontic tooth movement necessitates substantial remodeling of the periodontium. Remodeling is believed to begin in the periodontal ligament. As a biological exudate, gingival crevicular fluid may be quantified in order to detect particular biomarkers with appropriate sensitivity. Substances involved in bone remodeling have also been studied by measuring GCF during orthodontic tooth movement.[1] Orthodontic tooth movement is accomplished by a combination of osteoclastic bone resorption and osteoclastic bone formation. Osteoclastogenesis is influenced by a variety of factors, including cytokines, hormones, growth factors, and bone matrix elements. TNF-α is critical for osteoclast recruitment and activation, either directly or via chemokine release.[2] Cytokines, which can be pro-inflammatory or anti-inflammatory, and contribute to the initiation, amplification, perpetuation, and resolution of inflammatory reactions. Interleukins (IL-1,2,6 and 8) and Tumor Necrosis Factor are pro-inflammatory whereas Interleukins (IL-4,10 and 13) are anti-inflammatory cytokines.

Alarm cytokines are proinflammatory substances that promote inflammatory response by causing vascular dilatation and increased permeability. T-helper 1 cells produce the proinflammatory cytokine IL-2. This cytokine increases osteoclast activity, natural killer cells, macrophages. T-cell proliferation, and B-cell activation. Additionally, IL-2 has been linked to bone resorption's osteoclast activity activation.[3]Signaling molecules are responsible for changing the blood flow and vascularity within the PDL after mechanical stress. Arachidonic acid metabolites (Eicosanoids), neurotransmitters (substance P and calcitonin gene-related peptide) are the first messengers in the cascade, followed by second messengers such as phosphoinositol phosphates, cyclic AMP and diacyl glycerol.[4] Through vasodilation and leukocyte infiltration of the tissue, pro-inflammatory cytokines including interleukin (IL)-1beta, IL-2, IL-5, IL-6, IL-8, TNF-α and GM-CSF cause the characteristics signs of inflammation, whereas anti-inflammatory cytokines like IL-4 and IL-10 are implicated in its resolution. Activated monocytes are the major source of IL-1 beta release, which then takes part in the acute phase response to trigger bone resorption.[5]

Gingival Crevicular Fluid (GCF) analysis may be regarded as valid method of illustrating the biochemical changes occuring during orthodontic tooth movement.

Core elements in the regulation of orthodontic forces: The success of orthodontic therapy depends on maintaining proper dental cleanliness, maintaining good periodontal health, and using the right amount of force. When orthodontic force is used to move teeth, the dental and periodontal tissues undergo remodelling changes. The periodontal tissue remodelling, which include the dental pulp, periodontal ligament (PDL), gingiva and alveolar bone is one of two connected processes that contribute to orthodontic tooth movement (OTM). The other is bone bending. The PDL and the alveolar bone are compressed (pressured) on one side of the force being applied, while the PDL is stretched on the opposite side (tension). The vascularity of periodontal tissues is altered by orthodontic stresses, which triggers the production of a number of signalling molecules and metabolites. Around the teeth, the released chemicals cause cellular reactions that create a favourable microbiological environment for tissue regrowth or degeneration. The activation of many cell signalling pathways promotes PDL turnover as well as localized bone resorption and bone deposition.[6]

PHASES OF ORTHODONTIC TOOTH MOVEMENT

Previous literature by Burstone reported three phases by which orthodontic forces can achieve tooth movement - Initial phase, Lag phase and Post lag phase.[7,8] However, a study by Pilon et al. has established four phases of tooth movement on the basis of the curve of tooth movement which is described in table-1.[7]

BIOMARKERS INVOLVED IN ORTHODONTIC TOOTH MOVEMENT: A biomarker are substances that are objectively detected and assessed as a sign of healthy biological activity, pathogenic processes, or pharmacological reactions to therapeutic interventions. A viable biomarker must be precise, sensitive and able to convey the biological status in terms of periodontal tissue alterations and how those alterations relate to a certain OTM phase. Knowing the sort of cellular process can help you deliver the right mechanical loading and cut down on treatment time, which can also help you prevent side effects from orthodontic therapy.[9]

Biomarkers can be divided based on:[4]

- Markers of Alveolar Bone Remodelling
- Markers in bone formation
- Markers of bone resorption
- Markers of Root Resorption

BIOMARKERS - THE KEY FACTORS IN ORTHODONTIC TOOTH MOVEMENT: An effective treatment is achieved by means of a detailed understanding of the underlying mechanisms of tooth movement for which the crucial role of bio-markers is essential. For the present narrative review, a computerized search was done in Pubmed, Google Scholar and other databases for English articles published since the last decade. The merit of this review is to provide a collective narration of the scattered literature on the various underlying mechanisms of orthodontic bio-markers influencing tooth movement in order to enhance an effective treatment plan for clinicians. Table 2 summarizes some of the significant orthodontic biomarkers which act as key factors in orthodontic tooth movement. The citations of the various biological markers are specified within the description of the current review.

Proinflammatory Cytokines: Interleukin-1β (IL-1 β), IL-6, IL-8, Prostaglandin E and Tumor Necrosis Factor-α (TNF- α). Neuroimmune interactions may play a major role in the first inflammatory response during experimental tooth movement. One of the cytokines, Interleukin-1β (IL-1β) is most effective in the early stages of orthodontic tooth movement in the periodontal environment. During tooth movement cells including macrophages, fibroblasts, osteoblasts, osteoclasts, cementoblasts, and cementoclasts are possible sources of IL-1β.[10] Many kinds of periodontal cells at 12 and 24 hours stained favorably for IL-1β in the initial stages of tooth movement. In particular, during the first stage of orthodontic therapy, osteoclasts immediately release IL-1β in response to mechanical stress, and macrophages later on, when their concentration has been seen in compressed regions, release IL-1β. Since IL-1β correlates with osteoclast survival, fusion, and activity, this interleukin also controls the degree of the alveolar bone remodeling process which subsequently affects tooth movement.[11] Within three hours of orthodontic force loading, there is a rise in IL-1β mRNA levels, particularly on the pressure side, in the periodontal ligament of rats.[12] Inflammatory cytokines and their corresponding receptors were expressed at greater levels in rats treated with orthodontics as a result of the buccal cortical plate being punctured during an inflammatory phase. Exogenous IL-1Receptor Antagonist (IL-1RA) therapy really results in a 66% reduction in IL-1β in treated mice in contrast to experimental tooth displacement in vehicle-treated animals. This finding suggests that, after histological characterization, on the pressure side of periodontal tissues, there are fewer osteoclasts, which when administered to mice receiving IL-RA treatment, reduces the incidence of orthodontic tooth displacement. The polymorphisms in the IL-1β gene cluster present in the GCF are also associated with the rate of tooth translation. It is believed that IL-1β is a potent inducer of the production of Interleukin-6 (IL-6); its effects overlap those of TNF-α and IL-6.[13] In inflammatory regions, it controls immune responses, and it stimulates the production of new osteoclasts and the activity of existing osteoclasts to break down bone. TNF-α is another pro-inflammatory cytokine that has been shown to induce acute or chronic inflammation and accelerate bone resorption by directly differentiating osteoclast precursors into osteoclasts in the presence of the Macrophage colony-stimulating factor. Interleukin -8 (IL-8) levels were found to be higher in PDL stress locations, and Tuncer et al. suggested that this could be a mechanism that initiates bone remodeling. Numerous studies have shown elevated amounts of these proinflammatory cytokines, which are involved in periodontal remodeling during orthodontic tooth movement, in the fluid of the human gingival crevicular.[14]

Osteoprotegerin (OPG) & RANK/RANKL: Numerous proliferation indicators are expressed as a result of orthodontic movement. In stress regions, Runx2, 3.6Col1-GFP, and BSP-GFP expression cells exhibit an increase in mature osteoblasts, whereas KI-67 and RANKL (receptor activator of nuclear factor-kappa B ligand) show an increase in osteoclast recruitment in compression areas. Notably, it was shown that TNF-related ligand RANKL, Osteoprotegerin (OPG), and its corresponding receptor, RANK (receptor activator of nuclear factor kappa B), are essential for the regulation of bone

metabolism.[15] Numerous cytokines and hormones affect osteo-resorption by producing and activating osteoclasts, a process that is controlled by RANKL.[16]It has an effect when RANKL interacts with the osteoclast cell lineage's RANK receptor. On the osteoblast cell lineage, RANKL is expressed. Due to this binding, hematopoietic osteoclast precursors quickly differentiate into adult osteoclasts. A counterfeit receptor called OPG that challenges RANK for RANKL binding, is produced by osteoblastic cells. OPG inhibits the latter phases of osteoclast formation, inhibits matrix osteoclast activation, and induces death in bone cells, among other biological actions. As a result, the balance between OPG and RANK-RANKL binding production determines bone remodeling.[14] Recently, Kanzaki et al. found that OPG gene transfer inhibits experimental rat tooth mobility and RANKL-mediated osteoclastogenesis in periodontal tissues. This finding highlights the potential advantages of combining biological agents with orthodontic treatment. Further, the prevention of anchor tooth movement during orthodontic treatment and a recurrence during the recovery phase may thus be greatly aided by the restriction of RANKL activity in its promotion of osteoclast development.[17]

Macrophages- Colony Stimulating Factors (M-CSF): Colony-stimulating factor (CSF), a group of specific glycoproteins, combine to modulate the development, maturation, and activity of granulocytes (G-CSF) and monocyte-macrophages (M-CSF). They could have an effect on bone remodeling and therefore on tooth mobility.[18]Due to its accelerated early osteoclastic recruitment and differentiation, the M-CSF has a considerable effect on tooth mobility. The future holds enormous promise for speeding clinically the pace of tooth movement thanks to appropriate M-CSF doses, which are already connected with measurably changing gene expression and tooth movement.[14]

Vascular Endothelial Growth Factor (VEGF): The cytokine-VEGF, which promotes angiogenesis and increases vascular permeability, is implicated in tissue neoformation.[19]When teeth are moved during orthodontic treatment, compression forces activate VEGF, which causes the periodontium to develop new blood vessels. Using immunohistochemistry analysis, the in vivo location of VEGF during prosthetic tooth movement in periodontal tissues of rats was determined. Numerous cells were found to be immunoreactive to VEGF, including fibroblasts, vascular endothelial cells close to hyalinized tissue, necrotic tissues adjacent to compressed zone, osteoclasts, osteoblasts and mononuclear cells. According to Kaku et al., during an experimental orthodontic tooth movement, fibroblasts and osteoblasts in the stress zone of the periodontal ligament in mice were observed to express VEFG mRNA.[20] These studies show how VEGF influences bone resorption and creation, as well as periodontal ligament remodeling.[14]

Neuropeptides: Biologically active protein concentration rises during orthodontic tooth movement, which results in periodontal neurogenic inflammation. Through somatosensory neurons, periodontal peripheral nerve fibers send impulses to the central nervous system. Calcitonin gene related peptide (CGRP) and Substance P are released by periodontal peripheral nerve fibers in response to the application of optimum orthodontic force. In addition to acting as CGRP, neurotransmitters and Substance P are vasodilators that encourage leukocyte movement into tissues, vascular diapedesis, and plasma extravasation (transmigration). Through osteoblast growth and osteoclast suppression, CGRP promotes bone production. The activation of CGRP receptors found on mast cells, lymphocytes, monocytes and osteoblasts are known to improve inter-cellular communication by promoting the production and release of cytokines (inflammatory mediator molecules). Periodontal remodeling brought on by orthodontic tooth movement requires normal periodontal and alveolar bone innervations. When teeth are moved in orthodontics, healthy innervation encourages optimum blood flow, whereas denervation inhibits bone growth. Substance P (SP), a unique sensory neuropeptide generated by sensory nerve peripheral terminals, has the power to influence how immunocompetent cells during the remodeling of periodontal tissue secrete pro-inflammatory cytokines. Notably, SP promotes the synthesis of PGE2.[14,21]

Enzymatic action at the level of Periodontium: Lactate Dehydrogenase (LDH), β-Glucuronidase (βG), Caspase-1, Aspartate Aminotransferase (AST) are various enzymes. A greater level of enzyme activity frequently reflects a high amount of cellular activity.To eradicate the hyalinized periodontal tissue that is generated during the early stages of orthodontic therapy, an apoptotic process occurs. Caspase-1 is the most essential of the apoptotic response mediators induced by intracellular ionic environment changes. Its function includes the processing and activation of prolL-1β and other pro-inflammatory cytokines. Caspase-1 mRNA expression is elevated in an orthodontic rat model, and its level fluctuates with distinct temporal stages of tooth movement. According to several studies, patients with diseases like rheumatoid arthritis may have a pathologic overexpression of the caspase-1 enzyme, which can lead to deformation of the periodontal tissues and irreversible root resorption. In order to maintain the structure of the periodontal ligament, it may be possible to provide molecules like VX-765 and pralnacasan that have the ability to suppress caspase-1 activity.[22]The lysosomal enzyme β-Glucuronidase (βG) indicates primary granule release from PMN leukocytes. This enzyme is found in elevated concentrations in the GCF of teenagers treated with a rapid palatal expander. βG, like other biochemical mediators such as IL-1 responds to both direct as well as indirect mechanical stress applied to teeth with a raised level that is higher than what happens after high pressure.[14] AST and LDH activities in GCF have been measured to support the biological activity that occurs in the periodontium during orthodontic therapy. These are soluble enzymes that are typically contained within the cytoplasm of cellular structures and released into the extracellular environment only in the event of cell necrosis. By the end of 1st and 2nd week, the AST activity in GCF is noticeably higher in both compression as well as tension zones. This rise can be explained by a controlled trauma that causes cell death as a result of mechanical forces on the alveolar bone and periodontium. The use of orthodontic force on a tooth structure, especially in the region of pressure is reflected in a minor rise in GCF AST activity, but an occlusal trauma result in a greater quantity of enzymatic level. It has a strong connection to the compression sites which is further emphasized by orthodontic tooth movement.[23]

Enzymatic action at the level of Alveolar bone: The enzymes Acid phosphatase (ACP) and Alkaline phosphatase (ALP) are involved in bone cell activity. Changes in the surrounding bone architecture are the last biological reaction to orthodontic tooth movement.

Table 1. Illustrates theunderlying mechanisms associated with the phases of orthodontic tooth movement[7]

	Phases of tooth movement	Changes seen at the level of periodontium	Changes seen at the cellular level
Phase I	Initial after force application	PDL width reduced on pressure side.	•Acuteinflammatoryresponse •Vasodilation. •Migration of leucocytes. •Release of cytokine-cell signalling molecules.
Phase II	0-35 days period of arrest	Hyalinization areas appear depending upon force.	•Chronicinflammation. •Continuation of migration ofleucocytes. •Paradentalremodeling.
Phase III	acceleration phase	•Continuous tooth movement with an increasing rate. •Remodeling of the periodontal ligament and alveolar bone reach their maximum capacity.	A subsequent acute inflammatory episode overlaid the ongoing chronic inflammation.
Phase IV	Linear phase	•Independent of force magnitude •Bone remodeling and periodontal ligament turn-over takes place at a more or less constant rate.	Recruitment of macrophages, fibroblasts, osteoblasts, and osteoclasts alkalinephosphatase activity.

Table 2. Summarizes the various biomarkers essential for orthodontic tooth movement

S:NO	BIOMARKERS	MECHANISM OF ACTION
INFLAMMATORY MEDIATORS		
1.	•Prostaglandin E2 •Substance P (neuropeptide) •Epidermal growth factor	Resorption of bone.
2.	•Transforming growth factor •Interleukins-1β, 2, 6, 8	Remodeling of bone.
3.	•Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL)	Stimulation of osteoclastic differentiation.
4.	•Osteoprotegerin	Inhibition of osteoclastic differentiation.
5.	•Granulocyte macrophage colony stimulating factor (GMCSF)	Bone turn-over
6.	•Myeloperoxidase	Inflammation of associated structures.
ENZYMES		
7	•Acid phosphatase	Resorption of bone.
8.	•Alkaline phosphatase	Formation of bone.
9	•Aspartate amino transferase	Cellular necrosis.
10.	•Cathepsin B	Degradation of extra-cellular matrix.
11.	•Matrix metalloproteins (1, 2, 8)	Breakdown of denatured collagen.
12.	•β-glucuronidase	Marker of granule release.
Metabolic products of paradental remodeling		
13.	•Hyaluronic acid (GAG)	Indicator of breakdown of gingival tissue.
14.	•Chondroitin sulfate (GAG)	Indicator of breakdown of alveolar bone and PDL.
15.	•Pentaxrin-3 (TNF stimulated gene14)	Inflammatory marker.
16.	•Osteocalcin	Bone turnover.
17.	•Insulin growth factor	Regulators of cellular differentiation and apoptosis.
18.	•Dentin matrix protein	Resorption of bone.

The expression of ALP and ACP by osteoblasts and osteoclasts respectively are associated with the metabolism of bone. ALP is a common tetrameric enzyme that is found outside of cell membranes. In comparison to other connective tissues, the periodontal ligament has much greater levels of ALP activity.[24]When orthodontic force is applied, these enzymes produced in periodontium diffuse into GCF. Thus, in experimental research in rats and clinical trials in humans, changes in GCF phosphatase activity and remodeling of alveolar bone are closely associated in their activities. In order to recognize and detect the enzymatic changes that occur during the first stage of applying orthodontic force together with the initial and lag phases of tooth movement, previous literature evidence has suggested a 21-day orthodontic cycle. The ALP activity of a majority of patients was seen to peak on day 14 before rapidly declining by day 21. The loss of the hyalinized zone is responsible for the decline in activity. ALP is thought to play a role in the mineralization process in hard bony tissues due to the strong staining response provided by active osteoblasts and osteocytes. Only in close proximity to cells that synthesize matrix is there any enzyme activity in the bone matrix. The periodontal ligament's osteogenic cells respond to tensional pressures by maturing more quickly.

Under tension stress proliferation of collagen and fibroblast have been demonstrated to increase in the periodontal ligament. In contrast, ACP activity is greater in the compressed hyalinized zones of the periodontal ligament. Both the tension and pressure areas of the alveolar wall experience a late period of bone deposition (7-14) days. Due to enhanced acid phosphatase activity, resorption predominates during the early stages of a bone remodeling cycle; however, throughout the latter phase, resorption and deposition become synchronized. After seven days, when bone deposition starts, high levels of ALP have been seen; a notable peak is reached on day 14. It is clear that an increase in the rate of fibroblasts and osteoblasts in the tension zone is a precursor to bone formation. This quantity is the consequence of mitotic cell division, which results in an increase in the number of cells. According to histologic studies, cell proliferation takes place in marginal tensional zones within 36-50 hrs, lasting for about 10 days or even up to three weeks. On the compression side, there would be a high amount of osteoclastic activity and little to no osteoblastic activity, resulting in bone resorption.[14]

Interleukins: One of the pro-inflammatory cytokines produced by a variety of cell types, including fibroblasts, osteoclasts, and polymorphonuclear leukocytes (PMNs), is

called an interleukin (IL). Orthodontic stimuli lead to the time-dependent production of regulatory proteins or pro-inflammatory cytokines, such as interleukins, that are involved in the remodeling process of the periodontium.[25] Interleukins have been investigated as indicators to comprehend the cellular metabolic reactions related to orthodontic tooth movement. They are essential for both the mechanical stress-induced bone remodeling processes and the normal physiologic turnover of bone. During orthodontic tooth movement, interleukins such as IL-1 along with IL-6, IL-8 and other pro-inflammatory interleukins, have been found in the GCF.[10] The cytokine IL-1 is generated by activated monocytes, macrophages, neutrophils, B-cells, fibroblasts and epithelial cells. It has proinflammatory features and is a strong bone resorption activator. It contributes to matrix disintegration, wound healing, and the pro-inflammatory process. Numerous cells including epithelial cells, fibroblasts, endothelial cells, and alveolar macrophages generate and release IL-8 as a result of inflammation. This is a strong pro-inflammatory cytokine that is crucial for the activation and recruitment of neutrophils during inflammation. As a result, inflammatory cells such as neutrophils move from PDL capillaries to the inflamed area. A cytokine produced by macrophages and T cells is interleukin-6. A buildup in the number of IL-6, in connective tissue as a result of increased production or decreased release into the GCF may interfere with the repair of periodontal pockets. According to studies, IL-1b can promote bone resorption when teeth are moved during orthodontic treatment.[9,23]

Tumor Necrosis Factor- α: TNF-α a pro-inflammatory cytokine produced by monocytes and macrophages, has the ability to increase osteoclastic activity and the production of proteolytic enzymes. It is a distinct pro-inflammatory cytokine that has been studied in relation to orthodontic tooth movement and is attributed to both acute and chronic inflammation as well as bone resorption. Monocytes and macrophages that have been activated, along with osteoblasts, endothelial cells and epithelial cells are the main producers of TNF-α.[26]It may serve as a signal for osteoclast recruitment to resorb bone on the side that is being crushed by PDL pressure since it inhibits osteoblasts and functions as an apoptotic factor for osteocytes.[9,27,28]

Prostaglandin E2: Prostaglandin E2 (PGE2), an arachidonic acid metabolite and a strong biochemical mediator of inflammation, has a multitude of pro-inflammatory actions and the mechanism of bone resorption brought on by orthodontic tooth movement is known to be affected by it. The local cells are stimulated to create and release PGE2 when orthodontic tension is applied to the tooth, which then triggers osteoclastic bone resorption.[28]PGE2 production, which is partially regulated by IL-1, is believed to be an effective bone resorption stimulant. According to studies, the amount of PGE2 in the human GCF peaked 24 hrs after the application of mechanical force and returned to baseline after 7 days.[9,27,28,29]

Glycosaminoglycans: In normal conditions, glycosaminoglycans (GAGs) are negatively charged complex carbohydrates that are covalently bonded to a core protein in order to form proteoglycans. They can be found in connective tissues and the extracellular matrix of mineralization.[30] Studies have been done on the GCF-GAG alterations that take place before and after orthodontic treatment. Contrary to variations in the severity of gingival inflammation, which were correlated to increases in GCF volume during orthodontic movement and decreases during retention, the change in a glycosaminoglycan component, chondroitin sulfate, was related to the length of retention.[31] They came to the conclusion that changes in the deeper tissues of the PDL and alveolar bone following orthodontic therapy are likely represented by chondroitin sulfate, a GAG component of GCF. Samuels et al. discovered that various types of orthodontic tooth movement correlated with different GAG levels in their investigation of GCF during orthodontic therapy. During orthodontic tooth movement, the content of chondroitin sulfate in particular can be a signal to be paid attention to on.[9]

Granulocyte Macrophage Colony Stimulating Factor: Osteoclast recruitment can be triggered by the granulocyte-macrophage stimulating factor (GMCSF) and vascular endothelial growth factor (VEGF). They are thought to play a key role in the remodeling of the bone. It has been documented that during canine retraction, the VEGF and GMCSF increase that is present in the gingival crevicular fluid. Through osteoclastic bone resorption, these factors cause bone remodeling.[9,20,27,28]

Osteocalcin: A significant part of the extracellular matrix of bones is a non-collagenous matrix protein called osteocalcin (OC). It is made by osteoblasts and is thought to be the most accurate indicator of osteoblastic function.[32] It is thought to be involved in both mineralization and bone resorption because it structurally connects to the two main bone constituents, collagen and apatite. In the gingival crevicular fluid, osteocalcin is regarded as a marker for both bone resorption and bone turnover that begins during bone development. Osteocalcin is present in the gingival crevicular fluid of patients with periodontal disease, the increase in the osteocalcin content have been associated with high rates of bone turnover through promoting osteoclastogenesis.[33]

Osteoprotegerin, Receptor Activator of Nuclear Factor Kappa-B (RANK) and Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL): It is well known that the osteoclastic production from precursor cells that is essential for the process of bone remodeling is stimulated by RANK along with its receptor OPG and RANKL. Since RANKL binds to RANK, it has been suggested that during orthodontic tooth movement, pressure induces the formation and maintenance of osteoclasts. In order to stop osteoclasts from maturing and interacting with RANK with RANKL, osteoprotegrin, a dummy receptor generated from the osteoblastic lineage, must be present. A rise in the "RANKL- RANK" level and a decrease in the OPG level, both of which are suggestive of bone resorption, were seen in the 24 hours following the application of an orthodontic force.[34]

Transforming Growth Factor- β1 (TGFβ1): A multifunctional cytokine known as TGFβ1 mediates immune suppression, angiogenesis, apoptosis, extracellular matrix synthesis, and cell growth inhibition.[35]One of the most important cytokines with pleiotropic effects, TGFβ1 controls the inflammatory infiltrate by acting in both pro-inflammatiory and anti-inflammatory conditions. For mast cells, lymphocytes, neutrophils, and monocytes, this cytokine acts as a chemoattractant. Additionally, it triggers the production of proinflammatory cytokines by the aforementioned cells, including IL-1, IL-6, and TNF-α.[36]

Lactate Dehydrogenase (LDH): Exclusively after cell death, an enzyme that is found in the cytoplasm of cells and released extracellularly is Lactate dehydrogenase.

Orthodontic tooth mobility and LDH activity in GCF have been shown in studies to be related.[37]

Matrix Metalloproteinases: Enzymes known as matrix metalloproteinases (MMPs) are essential for both normal and pathological PDL remodeling. The collagenous extracellular matrix of the PDL and alveolar bone is altered during orthodontic tooth movement. Although the specific role played by the involvement of MMPs in osteoclastic bone resorption is unknown, bone resorption by osteoclasts entails acid demineralization of the inorganic matrix of the bone along with the destruction of the organic matrix of the by Cathepsin K. Collagenase -1 (MMP-1) and Collagenous-2 (MMP-8) are the enzymes that initiate the tissue remodeling due to their unique ability to break the triple-helical interstitial collagen. MMP-1 gene expression was shown to be elevated during the use of orthodontic force in dogs and to be reduced following its removal. MMP-1 was, however, imprecisely detected in the gingival crevicular fluid of patients undergoing orthodontic therapy.[38]

Acid phosphatase (ACP) and Alkaline phosphatase (ALP): Bone turnover is frequently measured by observing the activity of the enzyme ACP and ALP in the tissues. While bone growth is linked to greater alkaline phosphatase activity, bone resorption causes increases in acid phosphatase activity. Early tooth movement is typically accompanied by a rise in alkaline phosphatase, while later phases of tooth movement are typically accompanied by an increase in acid phosphatase.[1]

Aspartate Aminotransferase: Aspartate aminotransferase (AST), a soluble enzyme, is released from cells' cytoplasm to the extracellular environment during apoptosis. After 28 days in the GCF, greater AST levels are a reflection of bone remodeling and tissue deterioration that occur in the periodontium due to controlled orthodontic tooth movement.[39]

Cathepsin B: A multifunctional biomarker known as Cathepsin B (CAB), is an intracellular lysosomal cysteine proteinase, which is involved in the breakdown of extracellular substances protein and collagen turnover in the lysosomal system. When significant levels of cathepsin B are found within 24 hours of the start of orthodontic treatment, it is a sign that the clinical inflammatory process is taking place in the initial phases. The increase in CAB after 1 month after treatment indicates that exposed collagen fibers and collagen degradation by-products are being biologically broken down.[40]

β-Glucuronidase : β-Glucuronidase is a lysosomal enzyme that participates in the breakdown of connective tissue and is a sign of the release of primary granules from neutrophils. After activating an orthodontic appliance for two weeks, a considerable increase in β-glucuronidase has been seen.[9]

Interleukin-1 Receptor Antagonist (IL-1RA): IL-1RA inhibits the pro-inflammatory effects of IL-1β and modulates the immunological inflammatory response to orthodontic forces. IL-1RA can be quantified by Activity index (AI), which is the ratio of IL-1β and IL-1RA concentration in GCF. Faster orthodontic tooth movement is positively correlated with gingival IL-1RA expression, which is shown by a lower IL-1RA score.[41]

Interferon-gamma (IFN-γ): Interferon-gamma (IFN-γ), which is mostly generated by T-cells, is produced in greater quantities and is essential for the remodeling of the paradental system. Periodontium remodeling during orthodontic tooth movement could be linked to an immunoregulatory role along with bone modeling via Nitric oxide (NO) bone cell activation, which is a powerful activator of osteoclasts via RANKL activation and subsequent stimulation of bone resorption.[42]

Leptin: Leptin, is a cytokine and also a polypeptide hormone that enhances macrophage phagocytosis in response to the first stress or strain brought on by orthodontic forces. Following orthodontic therapy, there was evidence of decreased leptin levels in the gingival crevicular fluid as a result of cell necrosis in the PDL and tissue resorption.[43]

Insulin-like Growth Factor-1 (IGF-1) and Insulin-like Growth Factor-Binding Protein 3 (IGFBP-3) complex: Insulin-like growth factor-binding protein-3 (IGFBP-3) interacts with insulin-like growth factor-1 (IGF-1). The IGF-1/IGFBP-3 complex is critical in the remodeling of alveolar bone as a result of orthodontic pressures. Orthodontic stresses cause a decrease in IGFBP-3 secretion into GCF as well as changes in molecular structures. However, these alterations in IGFBP-3 have no connection with IGF-I binding, indicating that this binding protein plays an IGF-independent role in tooth mobility.[44]

Substance-P: A neuropeptide called Substance-P aids in the start of orthodontic treatment by aggregating and moving leukocytes to express a local immunological response at the location of orthodontic stressors. Additionally, it works to reduce the mental stress brought on by discomfort from the implanted appliance and promote osteoclast bone resorption activity. One day after the orthodontic device was installed, the research showed an increase in output in the test sites.[9]

Pentraxin-3: Acute-phase protein called pentraxin-3 (PTX3) is important in controlling the aseptic inflammatory response. As a result, within the first 24 hours after orthodontic treatment, it peaks quickly and then quickly declines on its own. As a result, it may serve as a possible early biomarker for orthodontic tooth movement.[9,23]

CONCLUSION

Studies have linked periodontium alterations to tooth mobility by using GCF to measure changes there. The bulk of these research, however, have only examined specific biomarkers, each of which is only a part of one biochemical pathway. Therefore, future study is recommended to take a set of GCF biomarkers into account in order to gain a better understanding. To more clearly show that GCF has an enormous potential as a diagnostic tool for monitoring clinical development in orthodontics, it is advised that future research concentrate on the improvement of GCF sample and measurement techniques as well as the connection between mediator generation and force reactivation.

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