Review Article

Biomarkers in Orthodontics: A Comprehensive Review

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Abstract

Biomarkers play a crucial role in understanding orthodontic tooth movement, bone remodeling, and periodontal health. This review explores various biomarkers associated with orthodontic treatment, including their significance in diagnosis, monitoring, and predicting treatment outcomes. The study discusses genetic, salivary, and gingival crevicular fluid biomarkers, as well as their relevance in pain perception and tissue remodeling. In addition, the review covers the types of biomarkers, dyes used for biomarker detection, and tests for biomarker analysis. By integrating biomarker analysis, orthodontic treatment can become more personalized and efficient. This paper aims to provide a comprehensive understanding of the types, mechanisms, and clinical applications of biomarkers in orthodontics, emphasizing their potential to revolutionize diagnostic and therapeutic approaches.

Keywords: Bone remodeling, Orthodontic biomarker dyes, Orthodontic tooth movement, Salivary biomarkers

Introduction

The field of orthodontics in dentistry deals with the monitoring, direction, and treatment of developing dentofacial structures. The foundation of orthodontic therapy is the idea that a tooth will move as the surrounding bone changes on the application of continuous pressure. The paradental and dental tissues are affected by the result of orthodontic tooth movement (OTM). Alveolar bone, gingival tissue, periodontal ligament (PDL), and other tissues are involved in remodeling and tooth pulp.[1] These tissues exhibit significant macroscopic and microscopic alterations in response to varied levels of mechanical loading, including variations in amplitude, frequency, and duration.[2] A biological marker is something that can be reliably measured and evaluated to reflect normal biological functions, disease processes, or the body's response to a treatment or medical intervention (NIH Biomarker Definitions Working Group, 2001). Hulka and colleagues refer to biological markers as cellular, biochemical, or molecular abnormalities that can be detected in biological samples such as human tissues, cells, or fluids (biomarkers).[3] Either the sick organ or the body's reaction to the illness produces the biomarker. Throughout the whole spectrum of the illness process, biomarkers may be helpful.[2]

A biomarker is a material that can be used to quantify and assess the typical biologic, pathological, or pharmacologic response to a treatment an excellent biomarker should be very specific and sensitive. By learning about the type of cellular process, the treatment time can be reduced. This will also facilitate the use of the ideal force level.[2]

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Materials and Methods

Study design

This review compiles data from multiple studies on orthodontic biomarkers, focusing on genetic, salivary, and gingival crevicular fluid (GCF) biomarkers. The study also incorporates research on biomarker testing methods and staining techniques used for visualization.

Data collection

Relevant studies were identified through searches in PubMed, Google Scholar, and other academic databases. The selection criteria included peer-reviewed articles and clinical trials published in the last two decades related to orthodontic biomarkers, biomarker dyes, and diagnostic testing methods.

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Biomarker classification

Biomarkers were categorized based on their function, including predictive, diagnostic, prognostic, and monitoring biomarkers. In addition, biomarker levels were analyzed at different stages of tooth movement.[4]

Types of Orthodontic Biomarkers (Table 1)

Predictive biomarkers

These biomarkers help determine whether an individual is more likely to experience a favorable response to a specific medication.

Prognostic biomarkers

These biomarkers provide insight into the likelihood of disease recurrence or progression over time.

Diagnostic biomarkers

These biomarkers are used to detect or confirm the presence of a disease or medical condition.

Susceptibility biomarkers

These biomarkers indicate the possibility of developing a disease in an individual who is currently healthy.

Pharmacodynamic biomarkers

These biomarkers identify and measure an individual's response to a specific medication, reflecting the drug's effects on the body.[5]

Monitoring biomarkers

These biomarkers are used to assess the current status of a disease or medical condition, tracking its progression or stability.[6]

Safety biomarkers

These biomarkers signal potential adverse effects, either before or after exposure to a medication, to help assess safety.

Methods of Detection

Genetic testing

Analyzing DNA samples to identify specific genetic markers that may influence treatment outcomes.[7]

Salivary analysis

Collecting and analyzing saliva to detect levels of inflammatory markers or enzymes related to periodontal health.

Radiographic evaluation

Using X-rays or cone beam computed tomography to assess skeletal and dental relationships.

Dyes for Biomarkers

Dyes play an essential role in biomarker research by highlighting biological processes for visualization.

Alizarin Red S

Used for identifying bone mineralization, this dye binds to calcium deposits in newly formed bone.

Tetracycline fluorescence

Tetracycline binds to calcium in bone and fluoresces under ultraviolet light, helping in visualizing bone remodeling during orthodontic treatment.

Hematoxylin and eosin (H&E) staining

A widely used histological stain, H&E helps identify tissue structures and inflammatory markers in orthodontic research.

Masson's trichrome staining

This stain is used to visualize collagen and extracellular matrix changes, helping assess tissue adaptation during orthodontic movement.

Tests for Biomarkers

To identify and measure biomarkers in orthodontics, several laboratory tests and diagnostic techniques are commonly used. These tests primarily focus on detecting specific proteins, enzymes, cytokines, or genetic markers that are involved in the biological mechanisms involved in OTM.[8]

Enzyme-linked immunosorbent assay

A widely used method for detecting and quantifying proteins such as interleukin (*IL*)-1β, prostaglandin E2 (*PGE2*), and matrix metalloproteinase-8 (*MMP-8*) in saliva and GCF.[9]

Table 1: Biomarkers in Orthodontic Tooth Movement[5]

Metabolic products of para dental remodeling	Inflammatory mediators	Enzymes and enzyme inhibitors
Glycosaminoglycans	Prostaglandin E	Cathepsin B
Pyridinium derivatives	Neuropeptides (Calcitonin related gene peptide and Substance P)	Acid phosphatase and alkaline phosphatase
Pentraxin 3	Transforming growth factor-α	Glucuronidase
N-telopeptide type 1 and osteocalcin	Epidermal growth factor	Aspartate aminotransferase
Matrix metalloproteins 1 & 8	α2-microglobulin and insulin-like growth factor-1	Lactate dehydrogenase
	IL1 receptor antagonist 1α, 2, 6, 8	
	Tumor necrosis factor	
	Macrophage-CSF	
	RANK/RANKL/osteoprotegerin system	
	Myeloperoxidase	
	Markers of root resorption	

Table 2: Biomarkers in orthodontic tooth movement, here's an outline of some of the commonly studied biomarkers, their roles, and their significance in the process of tooth movement

Author and year	Biomarker used in the study	Role/Function	Significance in orthodontic tooth movement
Yamaguchi and Mishima 2021[10]	RANKL	Activates osteoclastogenesis (bone resorption)	RANKL levels increase during tooth movement, contributing to bone resorption in the pressure zone.[7]
Nakamura *et al*., 1991[11]	OPG	Inhibits osteoclast differentiation by binding to RANKL	Counteracts RANKL, reducing osteoclast activity in the tension zone and preventing excessive bone loss.
	ALP	Bone formation marker (osteoblast activity)	Elevated ALP indicates active bone formation in the tension zone during tooth movement.
Griffiths *et al*., 1998[7]	Collagen type I	Major structural component of bone and dentin	Increased collagen synthesis indicates ongoing bone remodeling in response to orthodontic force.
Sorsa *et al*., 2006[12]	MMPs	Degrade the extracellular matrix, particularly during bone remodeling	MMPs play a role in extracellular matrix degradation during tooth movement, especially in the pressure zone.
Grieve *et al*.[13]	IL-1β	Proinflammatory cytokine that promotes bone resorption	Increased IL-1β levels in the periodontal ligament help mediate the inflammatory response during tooth movement.
Zainal Ariffin *et al*., 2011[14]	TNF-α	Proinflammatory cytokine that stimulates osteoclast differentiation	Elevated TNF-α levels support osteoclast-mediated bone resorption, aiding in tooth movement.
Lee *et al*., 2004[15]	PGE2	Involved in inflammatory response, regulates bone remodeling	PGE2 levels increase in response to mechanical force and modulate bone resorption and formation.
Last *et al*.[16]	TGF-β	Involved in cellular signaling and tissue remodeling	TGF-β regulates both osteoblast and osteoclast activity, influencing bone remodeling during tooth movement.
Pilon *et al*., 1996[17]	Runx2	Transcription factor essential for osteoblast differentiation	Runx2 is crucial for osteoblast activity and bone formation, particularly in the tension zone.

RANKL: Receptor Activator of NF-κB Ligand, OPG: Osteoprotegerin, ALP: Alkaline phosphatase, MMPs: Matrix metalloproteinases, IL-1β: Interleukin-1 beta, TNF-α: Tumor necrosis factor-alpha, PGE2: Prostaglandin E2, TGF-β: Transforming growth factor-beta, Runx2: Runt-related transcription factor 2

Polymerase chain reaction (PCR)

PCR detects genetic biomarkers such as *Runx2* and *COL1A1*, which influence bone remodeling and tooth movement responses.

Western blotting

Used to analyze protein expression levels, Western blotting confirms the presence of specific orthodontic biomarkers.

Mass spectrometry

A high-precision method for identifying and quantifying biomolecules, mass spectrometry is used to analyze complex protein interactions in orthodontic treatment.

Characterizes of biomarkers

- A biomarker should be straightforward to assess and safe
- Follow-up testing should be reasonably priced.
- A therapy that has been shown to alter the biomarker should be available.
- It needs to be the same for all sexes and ethnicities.
- The biomarker should have a high predictive value, be sensitive, and be specific if it is to be utilized as a diagnostic test.

Table 2 biomarkers in OTM, here's an outline of some of the commonly studied biomarkers, their roles, and their significance in the process of tooth movement.

Biomarkers in Pain and Tooth Movement

Orthodontic treatment induces mechanical stress on the PDL, leading to pain and inflammatory responses. Biomarkers play a critical role in assessing and understanding these responses.[18]

Table 3: Biomechanics of orthodontic tooth movement[23]

Phase	Duration	Key events and biological response
Initial	24H–2 days	Acute inflammation, vasodilation, cytokine release, leukocyte migration, paradental remodeling.
Arrest	20–30 days	Chronic inflammation, continued leukocyte migration, paradental remodeling.
Acceleration	40 days	Acute inflammation superimposed on chronic inflammation, increased tooth movement.
Linear	Overall movement	Recruitment of macrophages, fibroblasts, osteoblasts, osteoclasts; alkaline phosphatase activity.

Pain-related biomarkers such as Substance P, PGE2, and calcitonin gene-related peptide are involved in pain signaling and inflammation.[19] Elevated levels of these biomarkers in the PDL and gingiva correlate with orthodontic discomfort.

OTM occurs in stages: Application of force, inflammation, tissue breakdown, bone resorption, bone formation, and stabilization. Biomarkers such as RANKL, osteoprotegerin, and MMPs regulate osteoclast and osteoblast activity, controlling bone remodeling.[20]

Salivary and GCF biomarkers provide a non-invasive means to monitor pain perception and tissue changes during orthodontic treatment. Their continued research enhances personalized treatment strategies, reducing patient discomfort and improving treatment outcomes.

Table 4: Biomarkers in different stages of orthodontic tooth movement

Author and year	Biomarker	Initial phase (inflammation)	Lag phase (bone resorption)	Acceleration phase (bone formation)
Lader and Flanagan, 1998[24]	IL-1β	Very high	Moderate	Low
	IL-6	High	Moderate	Low
	PGE2	Very high	Moderate	Low
	TNF-α	High	Moderate	Low
Tanaka *et al*., 2011[25]	RANKL	Moderate	High	Low
	OPG	Low	Moderate	High
Kumar *et al*., 2015[26]	Osteocalcin	Low	Moderate	Very high
	ALP	Low	Moderate	High
	MMP-8	High	Moderate	Low
	MMP-9	Low	High	Moderate
	Osteopontin	Low	Moderate	High
	Collagen Type I	Low	Moderate	High

RANKL: Receptor Activator of NF-κB Ligand, OPG: Osteoprotegerin, ALP: Alkaline phosphatase, MMPs: Matrix metalloproteinases, IL-1β: Interleukin-1 beta, TNF-α: Tumor necrosis factor-alpha, PGE2: Prostaglandin E2

Biomechanics of OTM

OTM occurs through bone bending and periodontal tissue remodeling. When forces are applied, the PDL experiences compression on one side and tension on the other, triggering biological responses that facilitate movement.[21] This process involves cytokine release, osteoclast activation for bone resorption, and osteoblast activity for bone formation. The efficiency of OTM depends on force magnitude, duration, and individual biological responses.[19] Schwartz's pressure-tension theory explains that restricted blood flow on the pressure side leads to resorption, while the tension side promotes new bone formation. Biomarkers such as IL-1, IL-6, tumor necrosis factor-alpha, and PGE2 regulate inflammation and remodeling, while alkaline phosphatase indicates bone metabolism. GCF biomarkers like dentine sialophosphoprotein and lactate dehydrogenase help monitor root resorption and inflammation. Non-invasive saliva and GCF analysis aid in optimizing treatment outcomes and minimizing[22] (Table 3).

Biomarkers in Different Stages of OTM

OTM occurs in distinct phases: An initial inflammatory phase, a lag phase with bone resorption, and an acceleration phase where new bone is deposited.[12]

Biomarker levels fluctuate at each stage, indicating specific biological processes at work. Such as osteopontin is thought to promote or regulate osteoclast adhesion, attachment, and surface spreading during bone resorption.[24]

The table below summarizes the expression levels of various biomarkers during the different phases of OTM, along with corresponding citations [Table 4].

Orthodontics Implications of Biomarker

Orthodontic implications are as follows:

Enhanced diagnostic accuracy

Biomarkers can aid in early detection of potential complications and help in assessing the severity of malocclusions.

Predictive value

Certain biomarkers may provide insights into the rate of tooth mobility and the biological reaction of periodontal tissues, orthodontists can better predict treatment results.

Monitoring treatment progress

Regular assessment of specific biomarkers can offer a way to monitor the effectiveness of treatments in real-time, enabling timely adjustments to improve results.

Personalized orthodontics

By understanding individual variability in biomarker expression, treatments can be tailored to optimize effectiveness and minimize side effects.

Research and development

Ongoing research is essential to identify new biomarkers and validate existing ones, ensuring they can be reliably integrated into clinical practice.

Conclusion

Biomarkers in orthodontics hold significant promise for improving diagnosis, treatment planning, and the monitoring of patient progress. These molecular indicators can provide valuable insights into the biological mechanisms behind OTM, such as bone remodeling, tissue responses, and periodontal health. By identifying specific biomarkers, clinicians may be able to predict and address potential issues like root resorption or periodontal complications, leading to more personalized and efficient treatments.

As research into biomarkers continues to evolve, it is likely that these tools will become integral to clinical practice, offering better outcomes for patients. However, challenges such as standardization, validation, and ethical concerns must be addressed before their widespread use. Moving forward, advancements in genetic profiling and molecular imaging may enhance the ability to tailor orthodontic treatments even further.

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