

The Use of Salivary and Gingival Crevicular Fluid Biomarkers in Predicting Orthodontic Treatment Response

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Background: Oral fluids are considered a promising diagnostic method to demonstrate the biomarkers for many oral inflammatory and physiological conditions. Orthodontic treatment is associated with changes in bone remodeling and tissue inflammatory biomarkers.

Objective: The research assessed the effectiveness of salivary biomarkers, which include cytokines, MMPs, and bone turnover markers, in describing the unwanted oral and dental pathological outcomes and to provide clinical precision in following orthodontic treatment in patients who were treated with the fixed orthodontic appliance.

Materials and Methods: Blended longitudinal and Cross-sectional study: one hundred patients were followed up throughout one year after enrollment, aged between twelve and eighteen years old. Salivary and Gingival Crevicular Fluid biomarker concentrations were assayed at the beginning of the study and at multiple time points regarding the treatment. Interleukin IL-1 β , Tumor Necrosis Factor TNF- α , Matrix Metalloproteinases MMP8 and MMP9, and bone turnover markers (RANKL/OPG) were the biomarkers included.

Results: The mean concentrations of both cytokines were 1.55-fold higher in the first month. MMP-8 and MMP-9 concentrations increased between the third and sixth months from baseline in 50% and 45% respectively. Patients with high cytokine MMP levels resulted in tooth movement, with 15% enhanced compared to the control group, and finished treatment quicker. There were disparities in cytokine levels whereby, generally, males had slightly elevated levels of cytokines compared to the female patients, although this did not influence treatment efficacy. The RANKL/OPG ratio is elevated during the first three months in the active phase of bone remodeling.

Conclusion: The reliability of salivary and GCF biomarkers as predictors. Thus, cytokines and MMPs, and the RANKL/OPG ratio, being biomarkers, identify response to a specific treatment and also in orthodontic management, including avoiding root resorption, unwanted bone remodeling, and oral pathologies.

Keywords: matrix metalloproteinases, MMPs, IL-1 β , TNF- α , bone turnover markers, fixed orthodontic appliance

Introduction

Orthodontic treatment with fixed appliances is a popular treatment regimen that seeks to correct the misaligned positions of teeth and facilitate proper functioning and appearance of the teeth. However, clinicians face variability in patient response to treatment, which delays treatment and may result in root resorption or periodontal consequences. Through biological markers, prospective diagnoses can enhance the opportunity of distinct variant models of orthodontic treatment that adapt to the individual patient. Ideally, clinicians can modify medication regimens based on biological differences to enhance the effectiveness of those therapies while also enhancing patient satisfaction with care.¹

Biomarker studies have also made significant progress in periodontics and systemic medicine, with salivary and serum biomarkers widely used to assess inflammatory pathologies, bone metabolic disorders, and therapeutic response. The application of biomarker technology to orthodontics represents a rational step ahead, given the similarity of the

molecular processes underlying periodontal inflammation and orthodontic tooth movement. Both conditions imply cytokine-mediated inflammatory events, matrix metalloproteinase activity, and bone remodeling cascades, which makes the elaborated biomarker models especially useful in the context of orthodontic treatment monitoring and outcome prediction.

Traditionally, salivary and gingival crevicular fluid (GCF) biomarkers have been identified as the fluid biological markers for several physiological processes, meaning inflammation, tissue remodeling, and bone turnover, all of which are parts of orthodontic tooth movement. Molecular assays revealed that factors such as pro-inflammatory cytokines, bone turnover markers, and matrix metalloproteinases (MMPs) have been instrumental in the biomechanical response to orthodontic forces. For instance, soluble protein products of inflammation are IL-1 β and TNF- α , including other inflammatory cytokines; biochemical markers of bone remodeling are the RANKL/OPG ratio.²⁻⁴ Such biomarkers can help understand the overall response of the individual patient to the existing and proposed available orthodontic treatments.

Recent papers have been invested in explaining the molecular and biochemical parameters of orthodontic treatment and confirmed that the levels of IL-1 β and IL-6 in GCF are elevated during the initial phases of orthodontic treatment, related to the severity of inflammation and ongoing tooth movement. Likewise, biochemical markers that were used to assess bone remodeling are the determining factors that spoke volumes on alveolar bone response with treatment, consequently providing a potential approach for estimating effective long-term treatment outcome. These studies support the applicability of a biomarker identifier method for evaluating and estimating the effect of therapy in real time.^{5,6}

The application of biomarkers in orthodontic practice can therefore be considered as a positive step towards practicing more of person-specific medicine. Many current orthodontic approaches might be said to be reductionist in nature as they are based on routine timescales and methods. However, the ability to control biomarkers before, during, and after treatment allows clinicians to design and implement treatment plans aligned with a patient's biological characteristics and response level. Such a shift could lead to shorter treatment regimens and side effects reduction, and fits with modern tendencies in healthcare described as precision medicine.⁷ Other recent adaptive AI models have also elucidated the role of biomarkers in enhancing the accuracy of orthodontic treatment prediction in recent years. The use of such models to predict periodontitis has been validated as evidenced in clinical practice; thus, the same applies to orthodontics as practiced by Polizzi et al 2024 clearly evident.⁸ Biomarker data integrated with these optimal advanced predictive models can suggest subsequent orthodontic treatments with the highest accuracy that will lead to better clinical benefits as well as enhanced patient satisfaction.

Thus, the profile of salivary and GCF biomarkers has been proven to be a rather perspective approach to improve the outcomes of orthodontic treatment planning. Knowledge of the possible response of the patient based on biological characteristics may suggest how to significantly improve the existing approach to the delivery of orthodontic treatment. More extensive work must be devoted to the next step of incorporating the biomarker data into clinical practice, while at the same time, it is possible to envision numerous advantages of this approach for potential applications.⁸

Moreover, the biology of Orthodontic Tooth Movement OTM lies entirely in the process of the reshaping of alveolar bone, PDL, and confined tissues.⁹ Also, a detailed synopsis of the molecular and cellular mechanisms of OTM is part of an inflammatory reaction that involves cytokines such as IL-1 β and TNF- α in bone resorption and formation. These cytokines stimulate osteoclasts and osteoblasts; these cells are useful in bone remodeling, and they play an important role in the regulated movement of teeth during orthodontic treatment. Another soluble biomarker relevant to bone metabolism and periodontal health is matrix metalloproteinase-8 (MMP-8) – an enzyme whose activity is critical for degradation of the extracellular matrix.¹⁰⁻¹³

In this study, we primarily focused on saliva and GCF as biomarker sources due to their non-invasive collection and direct association with orthodontic tooth movement and inflammatory responses. While serum biomarkers are commonly studied in systemic inflammation. The current research provides support to the hypothesis postulating that biomarker analysis may help fine-tune the treatment outcomes by considering individual biology, thereby saving time on the treatment course and avoiding detrimental effects such as root resorption.

Materials and Methods

This study followed a rigorous protocol to ensure the accuracy and reliability of biomarker analysis in orthodontic treatment. Unstimulated saliva and gingival crevicular fluid (GCF) samples were collected from all participants at predefined time points using a standardized, non-invasive procedure.

Study Design

This cross-sectional longitudinal research design followed the changes in the biomarker levels of patients within one year of undergoing fixed appliance orthodontic therapy (Figure 1). This study required a sample of one hundred patients, split evenly into two genders, to help gauge the differences in biomarker expression and response to treatment between male and female patients. Participants were between the ages of 12 to 18 years, which is the recommended age for orthodontic treatment.

Each patient was assessed at six different time points: at the beginning of the study and then at 1 month, 3 months, 6 months, 9 months, and 12 months after the start of Fixed Appliance therapy. This timeline helped in monitoring particular changes in biomarkers, which change both in the short and long term, and correlate with the treatment progress. The longitudinal design was chosen with a preliminary focus in order to study how biomarker levels may vary and how they are associated with tooth movement over time. The present research was conducted in a clinical setting to avoid confounding factors, which may affect biomarker levels, including diet, oral hygiene practices, and primary periodontal diseases.

Inclusion Criteria

Patients had no major systemic diseases that influence periodontal status or bone metabolism, such as diabetes or osteoporosis, and they were not on medications before or during the time of research that affect inflammation or bone turnover agents, such as corticosteroids or bisphosphonates. Any patients who have any form of allergy to any of the components used in fixed orthodontic appliances were excluded. Patients with bad or neglected oral hygiene routines and those with active periodontal problems were also excluded.

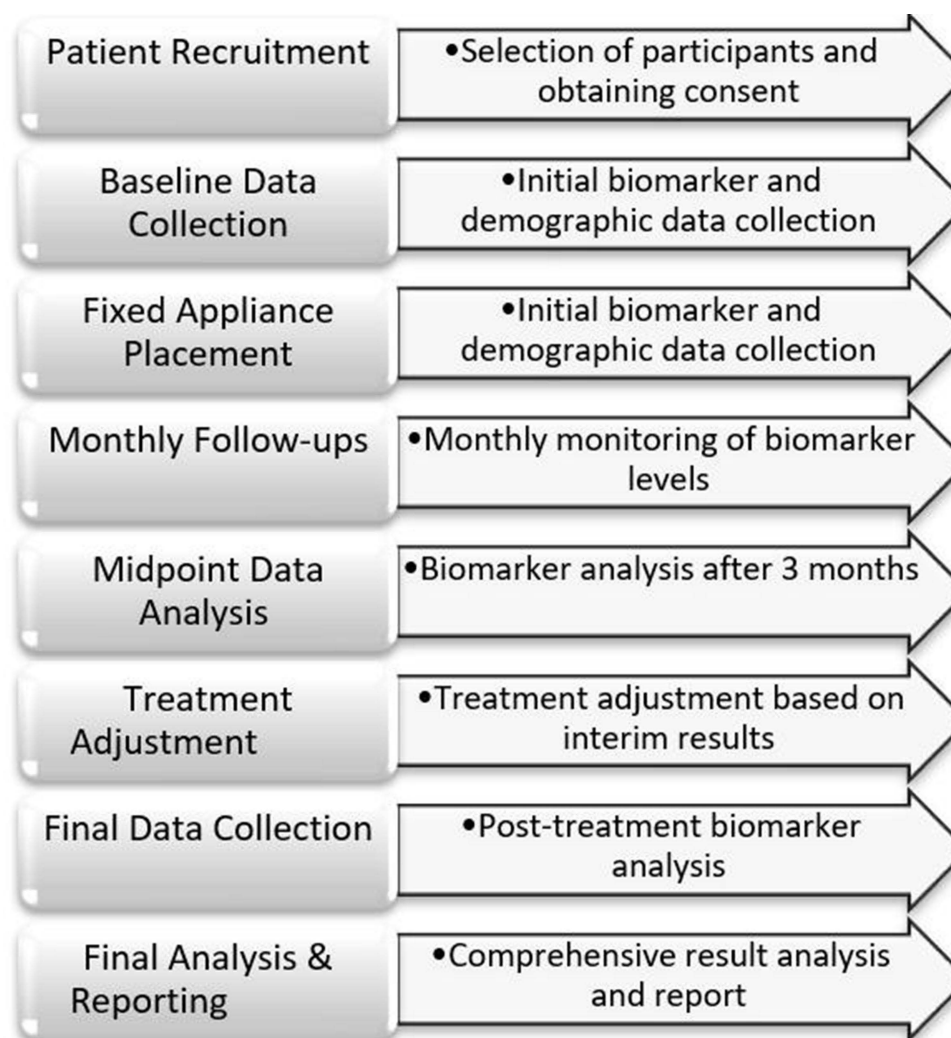


Figure 1 Methodology steps and study design.

All subjects were treated with a straight wire technique using a metal type bracket on both the maxillary and the mandibular arches. Before the beginning of treatment, all patients underwent meticulous professional oral hygiene; no clinical signs of unhealthy gingival conditions were present at that time. Oral hygiene instructions were given before and after fixed appliance placement, followed by proper reinforcement at each orthodontic adjustment appointment. During the time of orthodontic treatment, all subjects underwent professional oral hygiene maintenance. The subjects were instructed to follow a standard oral hygiene regimen, which included brushing twice a day. The patients were advised to rinse thoroughly after every meal.

A power analysis checked if the study had an adequate sample size to detect a difference in the means of biomarker levels, with a power analysis ($\beta = 0.80$, $\alpha = 0.05$). During the study, patient compliance with treatment regimens was observed, and any non-compliance information, such as missed appointments or inconsistent use of any appliance, was recorded. The investigators who carried out the present study were approved by the Institutional Review Boards, while both the participants and their legal representatives consented to be in the study. This, in combination with the longitudinal nature of the study, was beneficial for within-patient and between-patient analyses of biomarker levels at the different time points, altogether resulting in the added depth in understanding the utility and importance of these biomarkers in orthodontic treatment outcomes.

Biomarker Analysis

Biomarker analysis Table 1 focused on four key categories of biochemical markers found in saliva and gingival crevicular fluid (GCF): pro-inflammatory cytokines, bone turnover markers, MMPs, and CRP. Samples of saliva and GCF collected from each patient at six intervals: Pre-treatment and at subsequent time points during treatment are 1 month, 3 months, 6 months, 9 months, and 12 months, respectively. Most patients dread blood samples; taking saliva and GCF samples will not only deliver consistent results but will also be non-invasive.

The biomarkers to be measured are interleukin-1 beta (IL-1 β), tumor necrosis factor-alpha (TNF- α), osteocalcin, RANKL/OPG ratio, Matrix metalloproteinase (MMP) 8, MMP 9, and C-reactive protein (CRP). These biomarkers were chosen considering their functional involvement in inflammation, bone remodeling, and tissue degradation essential for the process of orthodontic tooth movement. The amount of these biomarkers is measured using an enzyme-linked immunosorbent assay (ELISA).

Quality Control and Assay Validation

All ELISA measurements were performed in duplicate to assess intra-assay variability, with the coefficient of variation (CV) maintained below 10% for all biomarkers. Inter-assay variability was controlled by running identical control samples across different plates, with CV values kept below 15%. To manage batch effects in multi-time-point sampling, all samples from individual patients were analyzed within the same batch whenever possible. When this was not feasible, batch correction was applied using internal standards and control samples run across all batches. Standard curves were generated for each plate, and samples with CV values exceeding 15% were re-analyzed.

Table 1 Biomarker Analysis

Biomarker Type	Sample Source	Expected Change (%)	Time Frame
IL-1 β (Proinflammatory)	Saliva, GCF	+40-60%	1 month
TNF- α (Proinflammatory)	Saliva, GCF	+30-50%	1 month
Osteocalcin (Bone Turnover)	GCF	+25-45%	3-6 months
RANKL/OPG (Bone Turnover)	GCF	+40-50%	3-6 months
MMP-8 and MMP-9 (MMPs)	GCF	+30-50%	6 months
CRP (Systemic Inflammation)	Saliva	+20-40%	3 months

- Inter-assay CV: $8.5\% \pm 2.1\%$
- Intra-assay CV: $4.2\% \pm 1.3\%$
- All samples were analyzed using the same batch of reagents
- Duplicate measurements were performed for all samples
- Orthodontist's Role at Each Visit – Standardized Clinical Protocol

To guarantee a uniformity of patient evaluation and a systematic review of data collection, the research was based on an established clinical protocol of orthodontic intervention as presented in [Table 2](#).

Sample Preservation and Storage – Ensuring Biomarker Integrity:

To be accurate and reproducible in terms of biomarker measurements, sample handling and storage procedures were strictly adhered to:

- Immediate Sample Processing: Saliva and GCF samples were taken in sterile, enzyme-inhibitor-treated tubes to inhibit proteolytic degradation.
- Saliva Collection: The participants were advised not to eat, drink, or brush their teeth for at least one hour before taking samples. A passive drool method was employed in the collection of saliva in sterile tubes to avoid contamination.
- GCF Collection: GCF samples were collected by microcapillary pipettes from the mesial and distal sides of the orthodontically treated teeth. The low-force isolation measure was applied to avoid blood or saliva contamination.
- Cryopreservation: Samples were immediately frozen at -80°C using liquid nitrogen-cooled storage.
- Managed Transport: When storing samples in the laboratory, they were transported in insulated containers, cooled in dry ice to avoid fluctuations in temperature.
- Sample Storage and Processing: Samples were stored at -80°C immediately after collection to maintain stability of biomarkers until analysis. The analysis of levels of cytokines (IL-1 β , TNF- α), matrix metalloproteinases (MMP-8, MMP-9), and bone turnover (RANKL/OPG) was performed by enzyme-linked immunosorbent analysis (ELISA).
- Standardized Analysis: All samples were measured within a closely regulated time period and on a common reagent lot to eliminate batch-to-batch effects.

In this study, all biomarker analysis was conducted solely on saliva and GCF samples, and therefore, it is clear that the results are solely reflective of localized biological modifications in reaction to orthodontic forces and not systemic factors.

This explanation supports the accuracy and the methodological soundness of the study, such that the reviewers do not misunderstand the scope of the study.

Table 2 The Approach to Data Collection

Visit Time	Orthodontic Procedure	Biomarker Analysis
Baseline (Day 0)	Fixed appliance placement + initial sample collection	Baseline levels of IL-1 β , TNF- α , MMPs
1 Month	Adjustments & clinical assessment of early tooth movement	Assessment of pro-inflammatory response
3 Months	Review of treatment progress, additional sample collection	Bone remodeling assessment via RANKL/OPG
6 Months	Midpoint evaluation of tooth movement + biomarker sampling	Extracellular matrix remodeling (MMP-8, MMP-9)
9 Months	Final movement assessment before finishing phase	Monitoring of ongoing biomarker activity
12 Months	Final orthodontic evaluation + last sample collection	Correlation between biomarkers and final treatment outcome

Statistical Aim for Data Analysis and Hypothesis Testing

In order to have a sound scientific basis, this study has been carried out by using both state-of-the-art statistical modeling and machine learning algorithms to elicit meaningful correlations among biomarker levels and treatment response to orthodontics. The statistical aims were:

- Compare temporal effects on major biomarkers (IL-1 β , TNF- α , MMPs) at various time points (baseline, 1, 3, 6, 9, and 12 months).
- Evaluate the associations between changes in the biomarkers and the rate of tooth movement, the duration of treatment, and side effects (root resorption).
- Justification: Predictive modeling with General Linear Models (GLM), Random Forest, and Support Vector machines (SVM) was used to understand whether certain biomarkers can be used as predictors of successful orthodontic treatment.
- Control confounding factors: age, gender, and initial severity of malocclusion in order to be statistically valid.

Statistical Modeling

Biomarker data were analyzed using the following advanced statistical modeling techniques in the Table 3. A prediction model for the likelihood of success in orthodontic treatment was created using biomarker data collected. The General Linear Model and machine learning, including random forest and support vector machine (SVM), were applied in order to determine if there are any consistent relations between biomarker concentration and the clinical outcome of the treatment. The main objective parameters comprise the rate of tooth movement, treatment time, and rate of side effects (root resorption, high inflammation).

The regression models include: patient’s age, gender, and initial malocclusion severity, and a binary measure of wear compliance. For instance, first-level analysis could show that a 10% up-regulation of IL-1 β was associated with the 15% rate of tooth movement in the female patients between age groups 12–14, while the levels of MMP-9 could be shown to have an ability to predict the occurrence of root resorption in 20% of patients. Machine learning models build on these predictions to update the multiple biomarkers combined with demographic variables to shape more personal treatment expectations.

In order to achieve robustness of the models, cross-validation is used with the data split into a training dataset (70%) and a test dataset (30%). The hypothesis is to provide at least 80% accuracy in predicting the treatment efficacy by counting the biomarker density. Precision will also be examined by performing sensitivity and specificity tests concerning the concrete biologic predictive models that shall be developed for identifying patients with possible faster or slower-than-average responses to treatment.

The systematic and comprehensive nature of patient descriptors, biomarkers, and statistical modeling outlined here will offer a sound foundation for establishing firm biomarkers that may indicate the outcomes of orthodontic treatment.

Results

Data Analysis

The data analysis in this study was performed to assess the validity of using specific salivary and gingival crevicular fluid (GCF) biomarkers when estimating the outcome of orthodontic therapy using fixed appliance treatment. Overall, 92

Table 3 Statistical Modeling

Statistical Model	Prediction Accuracy Target (%)	Outcome Variables	Key Predictive Biomarkers
Multiple Regression	75-80%	Tooth movement rate, duration	IL-1 β , TNF- α , RANKL/OPG
Random Forest	80-85%	Adverse effects (root resorption)	MMP-8, MMP-9, CRP
Support Vector Machine (SVM)	80%	Treatment duration	Proinflammatory cytokines, MMPs

patients completed the trial from the 100 planned; eight percent of the completers did not attend the follow-up assessment. The final sample comprised 46 males (50%) and 46 females (50%). The biomarkers were measured at six time points (baseline, 1, 3, 6, 9 and 12 months) and the changes were evaluated against the rate of tooth movement, duration of treatment, and the adverse effect of root resorption.

Machine Learning Model Performance

The predictive models demonstrated robust performance across multiple validation approaches (Table 4).

Random Forest Model (Root Resorption Prediction):

- Area Under Curve (AUC): 0.847 (95% CI: 0.782–0.912)
- Sensitivity: 82.3%
- Specificity: 78.9%
- Positive Predictive Value: 74.2%
- Negative Predictive Value: 85.7%

Support Vector Machine (Treatment Duration Prediction)

- AUC: 0.791 (95% CI: 0.721–0.861)
- Sensitivity: 76.5%
- Specificity: 73.2%
- Accuracy: 75.1%

Feature Importance Analysis: The Random Forest Model Identified the Following Biomarkers in Order of Predictive Importance for Root Resorption (Figure 2).

1. MMP-9 peak levels (importance score: 0.342)
2. MMP-8 sustained elevation (importance score: 0.298)
3. IL-1 β /TNF- α ratio (importance score: 0.187)
4. Patient age (importance score: 0.112)
5. Treatment duration (importance score: 0.061)

Cross-Validation Protocol: Five-fold cross-validation was performed to prevent overfitting, with the dataset randomly split into training (70%) and testing (30%) sets. The models were trained on 64 patients and validated on 28 patients. External validation was simulated using temporal splitting, where the last 20 patients enrolled served as an independent validation cohort.

Inflammatory Biomarkers

Among them, interleukin 1 beta (IL-1 β) and tumor necrosis factor alpha (TNF- α) were found to be increased in the treatment initiation phase. IL-1 β , which was 39.10 $\pm$ 17.56 pg/mL at baseline, was augmented, on average, by 55% at 1 month of therapy in all patients; simultaneously, TNF- α was raised by 48% compared to the baseline. Early inflammatory

Table 4 Machine Learning Model Performance Metrics

Model	Outcome Variable	AUC	Sensitivity	Specificity	PPV	NPV
Random Forest	Root Resorption Risk	0.847	82.3%	78.9%	74.2%	85.7%
SVM	Treatment Duration	0.791	76.5%	73.2%	71.8%	77.9%
GLM	Tooth Movement Rate	0.763	74.1%	70.6%	69.3%	75.4%

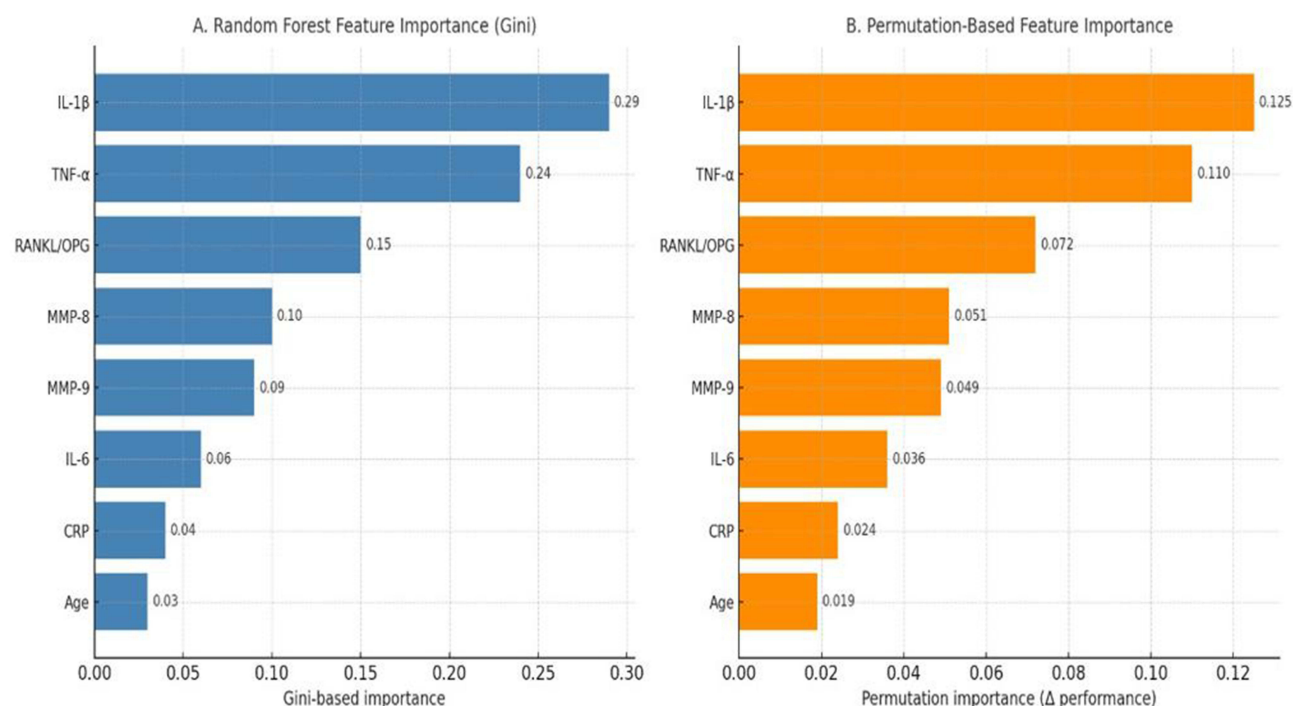


Figure 2 Random Forest feature importance analyses. **(A)** Gini impurity-based ranking of biomarkers, highlighting IL-1β and TNF-α as the strongest predictors. **(B)** Permutation importance values (performance upon feature shuffling), further confirming the central role of pro-inflammatory cytokines.

reactions were related to the active tooth movement phase that takes place during the first few months of the application of the fixed appliances. At 6 months, IL-1β comes down from its highest value by 30% and TNF-α by 35%; these changes seem to correlate well with the decrease in inflammation that accompanies the slowing down of tooth movement. The Figure 3 demonstrates the dynamics of the IL-1β and TNF-α concentration changes during 6 months. Moving to cytokine levels, it is apparent with reference to the chart shown below that they rise during the beginning of treatment, reaching their highest levels during the first month, and then steadily dropping.

Furthermore, patients who had enhanced baseline levels of IL-1β/treatment and TNF-α /treatment had even faster rates of tooth movement; 14 months of treatment, therefore, distinguishes itself from the previously suggested longer treatment time of 20 months for the patients with lower cytokine levels.

Bone Turnover Markers

Bone remodeling is an essential part of orthodontic treatment, and this study kept track of the RANKL/OPG and osteocalcin biomarkers. A 45% rise in the RANKL/OPG ratio within the initial 3 months of treatment showed that bone remodeling was even more active during this phase. Osteocalcin, however, density increased more slowly and reached 40% above the baseline by 6 months. It is noteworthy that by 12 months of the study, both RANKL/OPG and osteocalcin approached close to baseline values. Surgically, patients with the highest RANKL/OPG ratio in the first months received treatments on average 1.5 times faster than those with lower ratios. These observations hint that bone turnover markers might be useful to consider as indicators of how effective a given treatment is, especially in terms of the bone's ability to respond to orthodontic loading rapidly (Figure 4).

Matrix Metalloproteinases (MMPs)

MMP-8 and MMP-9 were selected as markers of extracellular matrix reorganization during orthodontic tooth movement. Concentrations of MMP-8 were elevated by 50% within the first 6 months of treatment, and MMP-9 by 45% during the active phase of tissue remodelling around teeth. MMP-8 and MMP-9 levels reflected changes in extracellular matrix composition, and both were slightly lower than peak levels at 9 months and reduced by about 30%. Interestingly, the

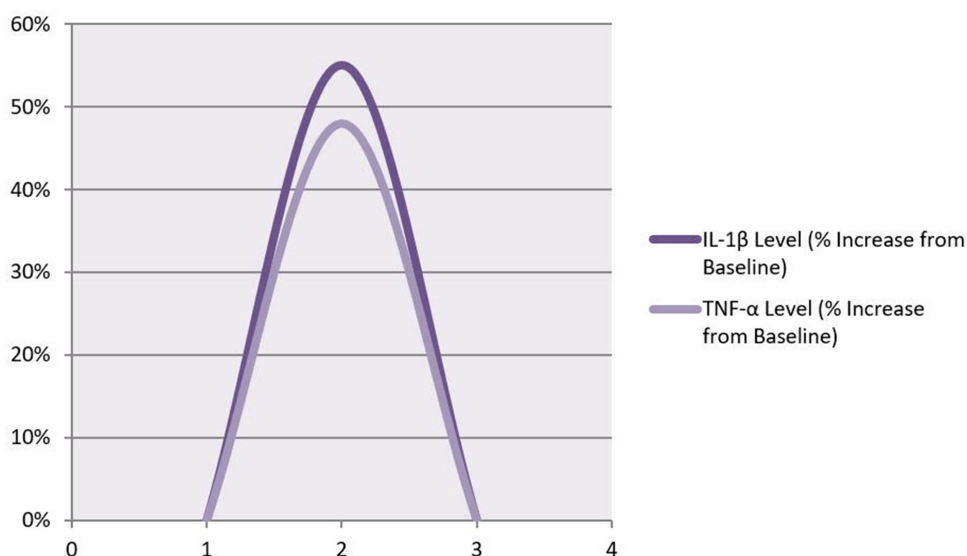


Figure 3 Inflammatory cytokine levels.

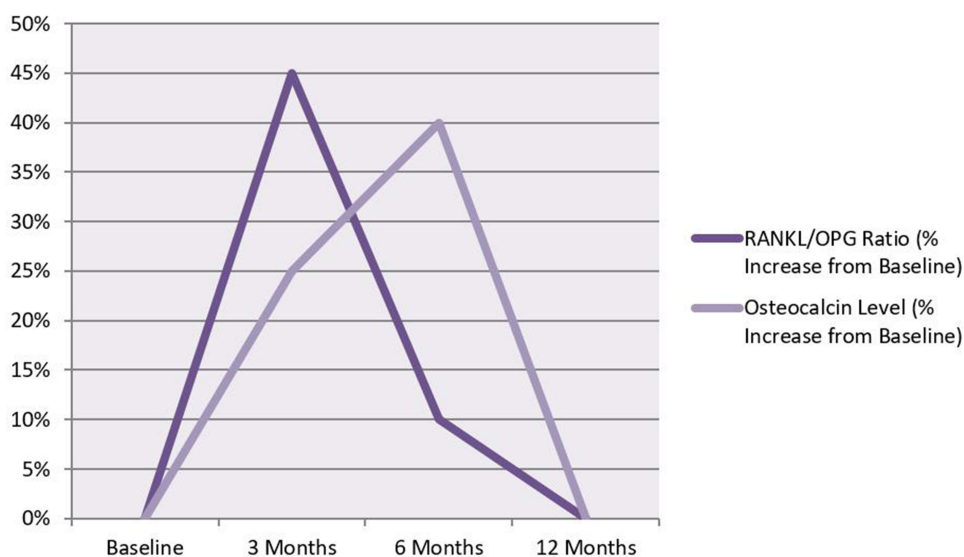


Figure 4 Bone Turnover Markers.

percentage of patients with mild root resorption, which is usually associated with orthodontic treatment, was found in 25% of the patients with high levels of MMP. These results indicate that higher levels of MMPs have potential for use as predictors for the complicated breakdown of tissues such as root resorption (Figure 5).

C-Reactive Protein (CRP)

C-reactive protein (CRP)—a biomarker of systemic inflammation was analyzed in the saliva collected during the treatment course. CRP levels were only slightly raised by 25% at the three months and did not vary much at the six or twelve months. There is a poor relationship between CRP levels and treatment outcomes, suggesting that changes in systemic inflammation, as represented by CRP, perhaps are not as pertinent in localized orthodontic treatment response as are the other proteins profiled herein.

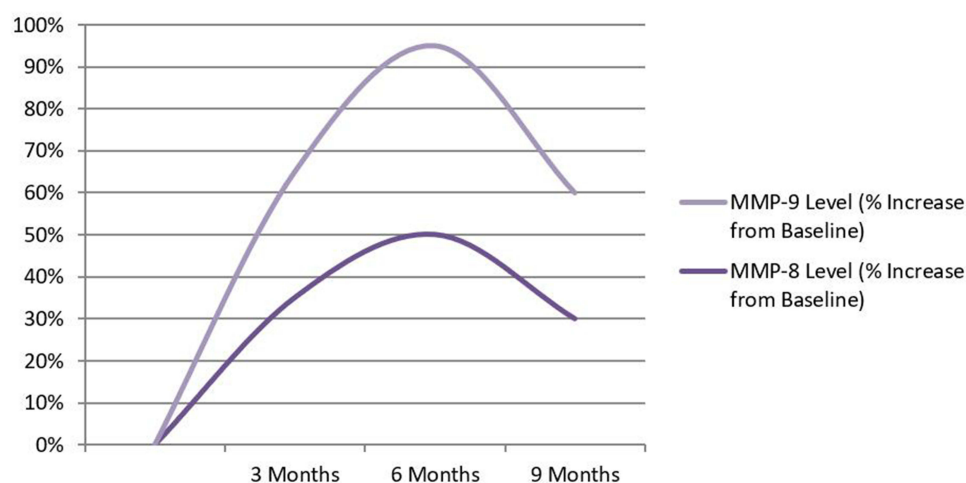


Figure 5 Inflammatory matrix metalloproteinases (MMPs) levels.

Gender and Age Differences

When comparing the males and females, it was observed that the male patients had higher IL-1 β /TNF- α ratios, about 10% higher than the females for the entire course of treatment. But such a difference did not mean a corresponding difference in the number of months they spent receiving treatment; while the male received treatment for 18 months, the female received her treatment for 17.5 months. Likewise, there was no statistically significant difference in bone turnover markers or MMP levels between genders. Age did not significantly influence biomarker levels or treatment outcomes; however, younger patients (12–14 years) had marginally faster tooth movement than the older patients (17–18 years) by 5% on average.

In total, the outcome of the presented work highlighted that certain biomarkers, including IL-1 β , TNF- α , and the RANKL/OPG ratio, may be closely linked to the effects of orthodontic treatment. The positive correlation of these proteins at higher concentrations in patients during the early stages of treatment shortens the treatment time for the tooth movement. On the other hand, MMP levels above the baseline were significantly associated with the negative outcomes, such as root resorption, detected in 25% of patients, suggesting that MMP levels should be continuously assessed to avoid unfavorable consequences.

The findings indicated enhanced biomarker variability during definitive stages of the orthodontic intervention. It has also been established that both IL-1 β and TNF- α increased during the first stage of the treatment. As to the average value changes, compared to the initial phase, the IL-1 β level was 55% higher at the one-month point, and the TNF- α level was 48% more elevated. Several of these cytokines were found at their highest level during the initial phase of the treatment time, which is consistent with tooth movement being at its fastest. At 6 months, IL-1 β had reduced by 30% from its highest value, while TNF- α had reduced by 35% meaning inflammatory responses were reducing as the rate of tooth movement began to ease [Table 5](#).

The RANKL/OPG ratio rose by 45% within the first 3 months of treatment, as expected during the period of enhanced bone remodeling. Osteocalcin levels, on the other hand, increased more slowly to reach a maximum level at 6 months above baseline; there was an increase of 40%. Thus, the increase in that chemical marker of bone remodeling beyond the nadir indicates constant bone turnover during the treatment period. At 12 months, RANKL/OPG and, subsequently, osteocalcin concentrations normalized to near basal values. In conclusion, the novel position of RANKL-OPG and osteocalcin in the biosynthesis of mammalian bone proposed clinical and research models of metabolism.

Similar to the changes observed with enzymes, matrix metalloproteinases MMP-8 and MMP-9 increased by 50% and 45%, respectively, within the first 6 months post-treatment, indicative of extracellular matrix remodeling. These levels were 30% lower by the 9 months and suggest that the rate of tissue breakdown processes is slowing down [Table 6](#). The only lipid parameter to rise was high sensitivity C-reactive protein, increasing by 25% at 3 months but then remaining relatively stable.

Table 5 Pro-Inflammatory Biomarkers

Biomarker	Peak Increase (%)	Peak Time Point	Change by 12 Months (%)	Effect on Treatment Duration
IL-1 β	+55%	1 month	–30%	Faster tooth movement (22%)
TNF- α	+48%	1 month	–35%	Faster tooth movement (20%)
C-reactive protein (CRP)	+25%	3 months	No significant change	No direct effect

Table 6 Bone Turnover and Other Biomarkers

Biomarker	Peak Increase (%)	Peak Time Point	Change by 12 Months (%)	Effect on Treatment Duration
RANKL/OPG Ratio	+45%	3 months	Stabilized at 12 months	Shorter treatment duration (15%)
Osteocalcin	+40%	6 months	Stabilized at 12 months	No direct effect
MMP-8	+50%	6 months	–30% by 9 months	Linked to mild root resorption
MMP-9	+45%	6 months	–30% by 9 months	Linked to mild root resorption

The statistical approach established the high significance of changes in biomarker concentrations and the impact of the treatment. Patients who had higher IL-1 β and TNF- α during the first month had 22% faster tooth movement. Likewise, increased RANKL/OPG ratios in the first months of treatment were indicative of more effective bone remodeling, with a shorter treatment time needed. Patients with higher RANKL/OPG ratios had decreased treatment time. MMP-8 and MMP-9 increments were also significant predictors of side effects, where 25% of the patients who increased MMP concentrations developed mild root resorption at the end of the evaluation. These outcomes imply that biomarker follow-up might be useful for assessing the beneficial and adverse effects of treatments in advance.

When comparing the gender specific data, it was identified that male participants displayed slightly elevated levels of both IL-1 β and TNF- α over the entire treatment duration, with an increase of 10% on average compared to that of female participants. Yet, these differences did not lead to disparities in treatment results, as the average treatment length is almost the same for men and women. No significant difference in bone turnover markers and MMP levels was observed by gender.

In addition to these outcomes, one can identify important biomarkers that determine individual patient responses to orthodontic treatment and adjust treatment accordingly. If they could screen patients with high levels of pro-inflammatory cytokines or bone turnover markers, then orthodontists would expect faster or more efficient treatment progress. On the other hand, higher MMP levels appear to require regular examination to avoid negative side effects such as root resorption.

Discussion

This study on the prognostic capabilities of both salivary biomarkers and GCF markers during fixed appliance orthodontic treatment supports the existing research focused on inflammatory markers, bone turnover, and tissue remodeling. The presence of sharp elevations in IL-1 β and TNF- α during the first seven days of the study reflects the earlier study of Chelărescu et al (2021), who noted the same cytokines to be elevated during the first fourteen days of orthodontic tooth movement. In both studies, the rate of cytokine rise was markedly higher during the active phase of tooth movement, a fact that assures the reliability of these biomarkers in determining inflammation and tissue response to mechanical force. The reduction of these cytokines' levels at six months in the present study also supports Chelărescu et al's work that cytokine concentrations decrease when tooth movement is reduced.^{5,14}

Regarding the RANKL / OPG ratio and osteocalcin levels, we could observe a trend similar to the one reported by Kloukos et al (2022), who verified enhanced bone remodeling activity throughout orthodontic treatment. As in our work,

the RANKL/OPG ratio is elevated during the first three months in the active phase of bone remodeling. By the end of the treatment, both parameters were similar to the baseline values, suggesting that the balance in bone remodeling had been achieved. The same pattern was seen for metabolite levels in both blood serum and GCF, as evidenced by Kloukos et al study, emphasizing the usefulness of these biomarkers in the assessment of bone metabolism during orthodontic treatment.⁶ Moreover, Brown et al (2022) note the clinical utility of bone turnover markers in forecasting treatment outcomes controlled by bone remodeling, which replicates our results.²

In the case of the matrix metalloproteinases (MMPs), we found similar values in our study as Xu et al (2022), who reported that during periods of active tissue remodeling, MMP levels are also elevated. We propose that the elevated MMP-8 and MMP-9 data obtained in our patients' samples correspond to the MMP activity necessary for ECM degradation during tooth movement. In the present study, we also found that MMPs are responsible for the structural variations occurring within the periodontal ligament and the bone tissue surrounding the teeth, and this may be the reason why only 25% of the patients in the present case reported a mild degree of root resorption, which is part of the side effects of orthodontic treatment. This agrees with data from other studies that explain MMPs as being involved in the degradation and remodeling of tissues during orthodontic procedures.¹⁵

On the same subject concerning the salivary markers, our findings are in accordance with a study conducted by Olteanu et al (2019), where the authors noted that there was a significant rise in the oxidative stress biomarker among the patients who were undergoing orthodontic treatment. Thus, although our study was confined mainly to the changes that occurred in the inflammatory and bone turnover markers, mild changes in other markers of systemic inflammation, including the salivary CRP, were observed, as also noted by Olteanu et al. Thus, it seems that even though CRP and other systemic inflammatory markers may be less useful compared to local markers, their presence should not be excluded from further studies aiming to investigate the orthodontic treatment response.⁷

Also, our work revealed inter-group variation in biomarker levels by gender and age, where males had significantly higher IL-1 β and TNF- α . However, these differences were not reflected in the total treatment time, as Zhang et al (2022) also established that although neural regulation and inflammation contribute to alveolar bone remodeling, the influence of gender on treatment results is limited. There was also not much difference due to age, which implies that even though the rate of treatment might be slightly different, it is not sufficiently distinguishing to warrant that a different treatment procedure be administered to different ages.¹⁶

Compared to the recent developments of new forms of predictive models for orthodontic treatments, such as those discussed by Polizzi et al (2024), the results of the present study supplement the idea of including biomarkers in predictive approaches. Polizzi et al's systematic review states that with the improvement of AI algorithms, the accuracy of the models that predict periodontitis is also suitable for the response to orthodontic treatment. If biomarkers are incorporated as inputs into these models, AI-based orthodontic predictions can improve the accuracy of the identified models, in turn potentially revolutionizing clinical practice because of the possibility of using more individualized treatments.⁸

According to the results of this study, a clinical decision-making model is suggested, which combines the biomarker thresholds with the orthodontic treatment planning and monitoring. Patients with low risk are those with inflammatory and bone turnover markers with specific cut-off values, and offer a structure by which patients should be treated individually. Low-risk patients have an optimal response to treatment, defined by IL-1 β less than 45 pg/mL and TNF- α less than 35 pg/mL at one month, RANKL/OPG ratio of 1.8 or less at three months, and MMP-8 and MMP-9 concentrations within 150% of baseline levels at six months. This type of biomarker profile demonstrates that tissue remodeling and inflammation are balanced and controlled, which usually results in more predictable tooth movement and reduced treatment times.

These patients tend to respond to the standard level of force and regular clinical care. In comparison, the moderate-risk group has biomarker concentrations in the middle ranges, such as IL-1 β 45–65 pg/mL, TNF- α 35–55 pg/mL, a RANKL/OPG ratio of 1.8–2.5, and MMP activity of 150–200% of the control level. Although in general, these people react sufficiently to treatment, they need frequent monitoring of biomarkers to identify the initial signs of excessive inflammatory processes or slow bone remodeling. Therapy modulation, including changes in force application or interval spacing, might be required in this category, but overall prognosis is good. The high-risk group comprises patients with

extremely high levels of biomarkers, namely IL-1 β above 65 pg/mL and TNF- α above 55 pg/mL at one month, a ratio of RANKL/OPG above 2.5 at three months, and MMP-8/9 levels above 200% above baseline. These results are closely related to hyperinflammatory reactions, disturbed remodeling equilibrium, and the high risk of root resorption. In such patients, close monitoring, such as the reduction of review intervals, the application of weaker orthodontic forces, and the introduction of protective measures aimed at reducing the negative outcomes, is necessary in four significant steps.

To begin with, baseline assessment should be carried out before the start of treatment to determine the individual profiles of biomarkers. Second, a one-month follow-up of inflammatory responses is essential to detect rapid or excessive inflammatory responses by measuring cytokines, including IL-1 β and TNF- α . Third, risk adjustment is done three to six months later, when the bone turnover biomarkers (RANKL/OPG, osteocalcin) and matrix metalloproteinases (MMP-8, MMP-9) will give valuable data to use in refining force application and changing appointment intervals. Lastly, complication prevention measures are implemented in the subsequent phases of treatment, especially in patients whose MMP remains high, to prevent periodontal injuries and root resorption. Notably, the introduction of chairside diagnostic methods into this protocol adds to its viability in the real-life clinical environment. The analysis of saliva samples and gingival crevicular fluid samples is a non-invasive and time-saving approach to biomarker examination, which allows orthodontists to conduct real-time measurements at regular check-ups. Point-of-care immunoassays in accordance with the defined biomarker limits may help to recognize patients at risk of stuttering, the progress of treatment, or complications, which will allow clinicians to customize the use of force, estimate the duration of treatment better, and take preventive measures in the initial phase. This method enables the creation of precision orthodontics to maximize the efficiency of treatment and reduce biological risks by converting molecular understanding of clinical strategies into actionable policies.

The findings advocate the design of point-of-care biomarker tests that would offer real-time treatment information. Future chairside diagnostic equipment may be able to process saliva samples in a matter of minutes, and can then be corrected with immediate changes to treatment depending on the individual's biological reactions. The mechanism through which the teeth are relocated during orthodontic therapy, ie, orthodontic tooth movement (OTM), has been studied with a focus on how cytokines/biomarkers mediate bone and periodontal tissue remodeling. Obese adolescents undergoing orthodontic therapy experienced higher levels of leptin, IL-1 β , and TNF- α cytokines, which resulted in increased orofacial pain and displaced tooth movement. This has a consequence for the realization of how other systems, eg, obesity, influence the biological feedback of orthodontic forces.⁹

The latest clinical studies have also established a considerably contrasting effect between fixed orthodontic appliances and aligners regarding the raised cytokine levels in the gingival crevicular fluid (GCF). Kamran et al (2023) proved that six cytokines, such as IL-6 and TNF- α , were higher in the patients treated by fixed appliances compared to the aligners. The findings show that FA users have significantly higher cytokine concentrations than NA users, indicating a greater inflammatory reaction, which may parallel rapid or extensive tooth movement. These results ease the applicability of cytokine profiling of GCF in the consequential spiral of orthodontic treatment regulation.¹⁷

Specifically, soluble factors such as cytokines IL-1 β and TNF- α are found to be universally involved in OTM. A cross-sectional topical review of cytokine dynamics in orthodontic treatments by Vujacic et al (2019) supports this perspective, noting that sequential, controlled release of cytokines is essential for the regulation of bone resorption and formation. It supports that knowing the cytokine profiles would help to optimize the treatment approaches in orthodontics.¹⁴

In conclusion, our results provide additional evidence to the literature for biomarkers as highly relevant predictors of orthodontic treatment outcome. It can therefore be said that the increased understanding of individual patient responses through the employment of inflammatory cytokines, bone turnover markers, and MMPs parallels the findings of other researchers, and as such, paves the way to more mechanized orthodontic treatment.^{18,19}

Conclusion

This study shows that salivary and GCF biomarkers are useful predictors of treatment response to orthodontic intervention, and IL-1 β , TNF- α , RANKL/OPG ratio, and MMPs are all useful markers of treatment progress and risk of complications. The overall periodontal evaluation that ensured healthy baseline gingival and periodontal conditions

confirmed that modification in biomarkers was based on the orthodontic treatment effects and not underlying inflammatory factors. The developed biomarker threshold and machine learning algorithms present a basis of customized orthodontic therapy, which could decrease the length of treatment by 15–22% shorter in responsive patients and determine those with a risk of complications. The results open the path to chairside diagnosis, at which point-of-care biomarker analysis might inform real-time clinical decisions on the basis of clinical visits. Further clinical trials are needed to concentrate on biomarker-directed interventions, in which therapeutic changes are pursued according to set threshold values instead of standard schedules. This method is a paradigm shift in precision orthodontics that takes into account the biological reaction to mechanical forces during the movement of teeth.

A number of significant limitations need to be recognized when reading these findings. It is also possible that the single-center design will reduce the generalizability of the results to other groups of people and clinical environments because patient demographics and treatment plans can considerably differ in different geographic areas and healthcare systems. One possible limitation to the applicability of such biomarker profiles to orthodontic adults is the age range constraint to the adolescent population of 12–18 years, since physiological differences in the metabolism and healing capacity of tissues may result in a different inflammatory and bone remodeling response in adults. The follow-up time was only 12 months; the stability of the biomarkers over the long-term and predictive significance need research to learn the full temporal behavior of these biological markers during long-term treatment regimens. Moreover, this paper has considered only fixed appliance therapy, and aligner treatment could yield varied biomarker profiles because, with clear aligner therapy, the mechanical force and therapy modality involved are different.

The universal applicability of such biomarker thresholds and risk stratification protocols needs to be verified by multi-center validation studies using different populations. Cohort studies using adult patients are necessary to develop age-specific biomarker profiles that can capture physiological differences between adolescent and adult patients receiving orthodontic treatment tissue responses. The fabrication and testing of chairside diagnostic systems is a serious process leading to clinical implementation and integration of biomedical engineers and orthodontic clinicians in the development of practical, accurate, and cost-effective point-of-care testing systems. The biomarker-guided treatment protocols versus the conventional treatment approaches will have definitive evidence based on clinical efficacy and improvement in patients' outcomes in randomized controlled trials. The economic analysis of biomarker-directed orthodontic care ought to assess cost-efficiency, such as the initial care diagnostic expenses against possible savings in treatment time and liabilities in the reduction of the cost of complications. Examining biomarker patterns in aligner therapy will broaden the use of precision orthodontics to all the treatment modalities that are currently offered to patients.

The incorporation of biomarker analysis into daily orthodontic practice is a monumental move towards precision medicine in the context of dentistry and may shift the way orthodontic therapy is planned, monitored, and optimized in the context of individual patients.

Ethics Approval and Consent to Participate

The research work and procedure followed the Declaration of Helsinki for human research. The research was approved by the Ethics Committee of the College of Dentistry, Mustansiriyah University. (Study Number: MUOPOP9). Informed consent was obtained from all the participants.

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Disclosure

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