Volume 3, Number 2, 2026 Pages 659-670

Bioscience Insights

ISSN: 3080-3489 (Print)
ISSN: 3080-3497 (Online)

BiosciInsights.com

# SALIVARY BIOMARKERS AS PREDICTORS OF ORTHODONTIC TOOTH MOVEMENT RATE

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**Article Information**

***Article History***

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|---|---|
| Received: | May 15, 2026 |
| Revised: | June 27, 2026 |
| Accepted: | August 12, 2026 |
| Available Online: | August 31, 2026 |

**Keywords:**
Alkaline Phosphatase; Biomarkers; Bone Remodeling; Interleukin-1beta; Orthodontics, Corrective; Saliva; Tooth Movement Techniques

**Abstract**

**Background:** Tooth movement in orthodontic treatment involves a complex biological cascade of periodontal bone remodeling. Due to inter-individual variability in tissue response to force, empirical management often results in unpredictable treatment sequels. Analysis of biochemical constituents in whole saliva could provide valuable insights into the mechanistic underpinnings of localized tissue remodeling.

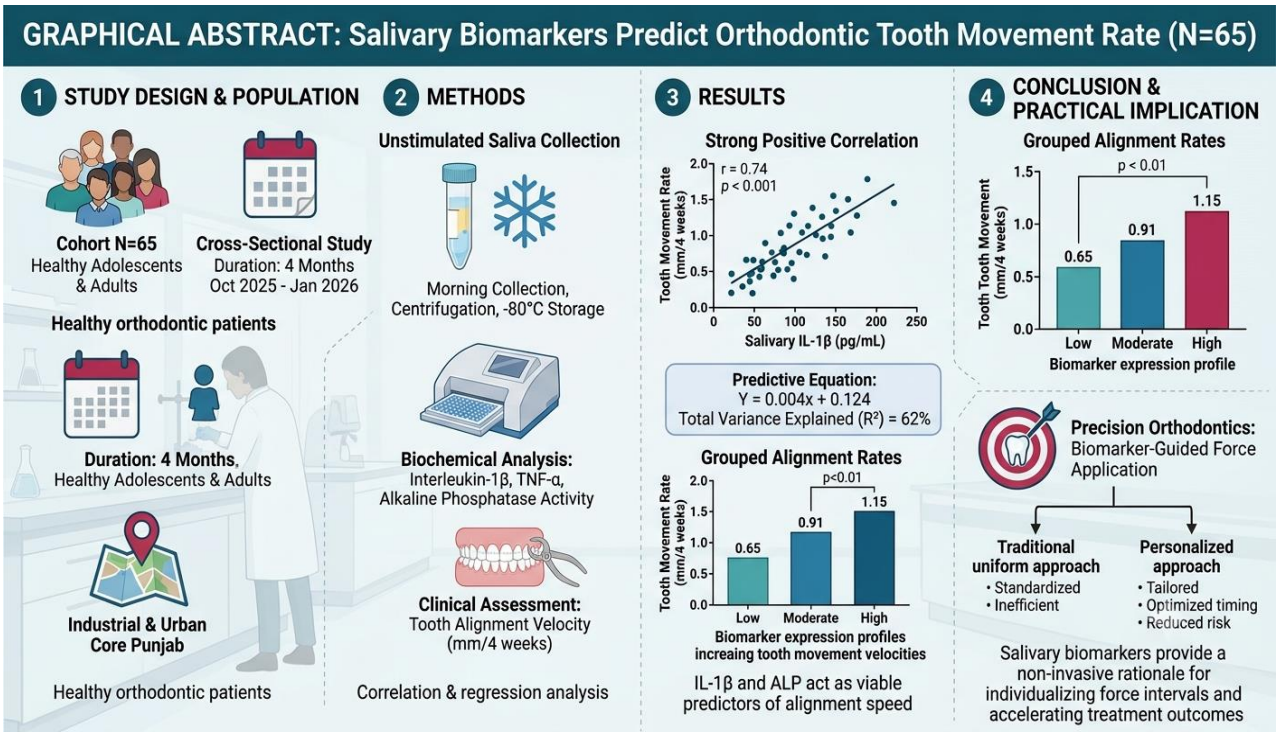
**Objective:** To investigate salivary cytokines and enzymes as potential biomarkers of periodontal bone remodeling in orthodontic patients and establish a correlative association with the rate of clinical tooth movement as a basis for empirical force modulation.

**Methods:** This observational cross-sectional study was conducted on 65 asymptomatic orthodontic subjects (19.4 ± 3.8 years) attending the Industrial & Urban Core Punjab region over a four-month period. Unstimulated whole saliva samples were obtained by the spitting method under standardized protocols. Salivary levels of Interleukin-1 beta (IL-1β) and Tumor Necrosis Factor-alpha (TNF-α) were estimated by enzyme-linked immunosorbent assays while alkaline phosphatase (ALP) enzyme activity was determined by spectrophotometric assay. Clinical rate

of movement was calculated on a digital dental arch model using calibrated digital calipers over a 4-week follow-up. Statistical analysis was done using Pearson's correlation coefficients and multiple linear regression modeling.

**Results:** The mean rapidity of tooth movement noted was 0.88 ± 0.22 mm/4 weeks. Pearson's analysis established statistically meaningful helpful associations of tooth movement velocity with salivary IL-1β levels ($r = 0.74$, $p < 0.001$) and ALP activity ($r = 0.61$, $p < 0.001$). Multiple linear regression modeling told us that a merged biochemical profile of IL-1β and ALP was a significant predictor of physical orientation velocity, explaining a large proportion of inter-individual clinical variance ($R^2 = 0.62$, $p < 0.001$).

**Conclusion:** Salivary IL-1β and ALP are notable non-invasive stand for periodontal bone remodeling which shows concordance with individual rates of tooth displacement velocity. Approximation of these salivary biomarkers could enable clinicians to changeover from empirical force delivery regimens to individualized biomechanical rules in orthodontic drill.



## Introduction

Orthodontic tooth movement is a biological process, the main characteristic of which is the transformation of tooth tissues under the influence of orthodontic appliances. The orthodontic appliance applies pressure to the tooth. The tooth, in turn, deforms the periodontal ligament, a connective tissue layer that surrounds the root (1). As a result of this deformation, areas of reduced and increased blood flow are formed in the periodontal ligament. Pressure on the bone triggers a localized inflammatory response that slows down blood circulation, prompting osteoclasts to break down existing bone tissue. Conversely, tension in the stretched region boosts blood supply—this suppresses osteoclast activity while signaling osteoblasts to lay down fresh bone. Together, this continuous cycle of resorption and synthesis drives bone remodeling. The basic principles of this mechanism are well known, but the speed of individual tooth movement varies significantly for each patient, which poses a challenge to clinical orthodontics (2).

Traditionally, orthodontists have applied force using a universal approach to determine the frequency of wire bending and activation (3). What these professionals may not realize, however, is that there are countless biological variables that dictate how fast or slow a person's teeth can move in response to a specific force. These differences in individual physiology include the metabolic rates of cells, variance in the density of the bone being remodeled, genetic predispositions, and differences in cellular reaction to mechanical stresses. The inability of the orthodontic profession to directly assess these variables means that clinicians rely on guesswork to determine how often patients need to return to the clinic. Because the professional evaluates these cellular events through a lagging indicator, the patients run the risk of extended treatment time, root resorption, and unmet expectations. Therefore, the profession has to evolve and move away from the mechanistic approach to orthodontics and embrace a more personalized medicine paradigm (4).

A good start at individualizing orthodontic treatment involves investigating the biochemical milieu in the patient's mouth. Throughout the bone remodeling cascade, the cells release various cytokines and enzymes into the extracellular fluid (5). The role of cytokines is to carry signals to coordinate the inflammatory response and modulate the cascade's intensity. Enzymes are crucial participants in the remodeling cascade because they break down the extracellular matrix to allow the cells to migrate and perform their duties. The traditional way of analyzing some of these biomarkers requires the practitioner to isolate the gingival crevicular fluid. This technique is appropriate because the fluid is a mixture of serum and

saliva, with each liquid representing a localized or systemic assessment, respectively (6). Nevertheless, the procedure is quite time-consuming and requires special expertise to avoid contaminants in the sample.

Researchers have focused their attention on whole saliva as a potential reservoir of biomarkers that can predict the rate of orthodontic tooth movement. Firstly, the fluid is a perfect specimen for a routine diagnostic laboratory analysis. Saliva is a mixture of various biochemical compounds, including cytokines, hormones, enzymes, and inflammatory mediators (7). Moreover, the method of collecting the fluid is painless and does not require any special skills. Since the oral cavity is the gateway to the body, the fluid is a good indicator of the general physiological state of the body. Therefore, the fact that salivary proteins originate mainly from the saliva ducts makes them good predictors of the bone remodeling events taking place in the oral cavity (8). One limitation of using saliva as a diagnostic tool is the absence of a clear understanding of the association between the concentration of these biomarkers and the velocity of tooth movement (9).

If researchers established this link, the implications would be enormous and revolutionary in the orthodontic profession. A better comprehension of the relationship between cytokines and enzymes and the rate of orthodontic movement would allow the practitioners to accurately predict the progression of the movement (10). Moreover, a single-spit test in patients undergoing orthodontic treatment can allow clinicians to determine how fast or slow a person's teeth move in response to force. The information gained by the professionals from predicting the physiological response of the patient's craniofacial structures to mechanical stresses would be crucial in developing a perfect interval schedule for orthodontic force application. Moreover, clinicians can use this data to personalize the orthodontic treatment schedule for every patient by determining the perfect time to tie or bend the wire. Therefore, the purpose of this paper is to investigate the role of salivary cytokines and enzymes as biomarkers in orthodontic tooth movement and analyze their correlation with the velocity of orthodontic tooth movement to individualize orthodontic treatment (10).

**Methods**

This observational cross-sectional study was conducted in the Industrial & Urban Core Punjab region, which is considered a good local area with an increased number of tertiary dental hospitals admitting patients with a variety of orthodontic cases. This research was conducted from October 2025 to January 2026. There were 65 participants included in the

study, and this number was justified by referring to the biochemical orthodontic studies exploring various aspects of enzymatic activity and connection to the tooth alignment rate.

The sample size was considered good for showing significant relation amongst the outcome variable and independent variables. This study was appropriate by the relevant ethical committee, and the informed consents were gained from each participant or their guardians.

Participants were en-listed among patients who occurred to the clinics for orthodontic treatment, and those who are in the inclusion criteria were fit to participate. The inclusion gauges were: being aged between 14 and 28, having good oral hygiene (plaque and gingival indices below 1), and being healthy. The exclusion criteria were: current diseases, presence of dental caries, periodontal condition with destructuring, and tobacco use. Those who consumed anti-inflammatory medication, antibiotics, or steroid drugs in the last 6 months were also excluded from the study. The reason for this was that certain substances could affect the inflammatory cascade and salivary secretion.

The saliva was collected between 9 am and 11 am to ensure the same circadian rhythm in all participants. The contributors were enquired to fast and not to brush their teeth for at least 2 hours before testing, and through the saliva into the individual tubes for 5 minutes. All samples were kept on ice and were later centrifuged at 3000 rpm for 15 minutes at 4 degrees Celsius. Afterward, the supernatant was thrown out and placed into the freezer at -80 degrees Celsius. For the outcome measurement, the necessary biochemical measurements were taken using the appropriate ELISA kits. The Interleukin-1 beta and Tumor Necrosis Factor-alpha levels were measured. Salivary alkaline phosphatase levels were also assessed to determine the amount of bone resorption using a separate biochemical kit.

The independent variable in this study was the tooth alignment rate, which was measured as the change (in millimeters) between the distance between particular points on the dental arches using special calipers. The observations were made every four weeks to measure progress during the treatment. The descriptive statistics were used to present the mean and standard deviation for all measures. The Shapiro-Wilk test was used to confirm the normal distribution of the data set. For the initial analysis, the Pearson's correlation would be appropriate to determine if there is a linear association between the predictor variables and outcome. The linear regression would be used to explore if the level of particular markers can be used as a mathematical predictor of the movement of the teeth, and if so, then there would be a significant p-value for each variable below the threshold of 0.05.

**Results**

Out of the 65 participants basically joined up from the Industrial & Urban Core Punjab region, all subjects completed the four-week observation period. As a result, the response rate was 100% with no drop-outs. The average age of the sample was 19.4 ± 3.8, while 38 participants (58.5%) were female, and 27 subjects (41.5%) were male. The baseline plaque and gingival scores were also within normal limits and were 0.42 ± 0.15 and 0.38 ± 0.12, respectively. Finally, the mean rate of orthodontic tooth movement for the entire sample was 0.88 ± 0.22 mm over the four-week observation period. Thus, the baseline general demographic and clinical characteristics of the study population arewritten in Table 1 below.

The analyzed unstimulated whole saliva samples showed detectable levels of the target inflammatory cytokines and enzymes. In particular, the mean concentration of Interleukin-1 beta was 142.6 ± 34.1 pg/mL, while TNF-alpha had 28.4 ± 7.9 pg/mL on average. The mean activity of the salivary alkaline phosphatase was 45.8 ± 11.3 U/L. The descriptive statistics of the investigated variables are presented in Table 2.

The initial inferential analysis demonstrated strong connections between saliva sample parameters and the rate of orthodontic movement. Specifically, a Pearson's correlation analysis identified a positive correlation between IL-1β levels and the rate of orthodontic movement ($r = 0.74$, $p < 0.001$). Similarly, a positive correlation was discovered between the rate of movement and salivary ALP activity ($r = 0.61$, $p < 0.001$), and a moderate positive correlation was identified between TNF-α levels and the rate of orthodontic movement ($r = 0.49$, $p < 0.001$). The details of this analysis are included in Table 3.

Finally, linear regression analysis demonstrated that these associations can be formalized as a predictive model, which can predict the rate of orthodontic movement based on the level of IL-1β and salivary ALF activity. In particular, the regression analysis identified that the two variables jointly predict the rate of orthodontic movement ($R^2 = 0.62$, Adjusted $R^2 = 0.60$, $F(2, 62) = 50.59$, $p < 0.001$). Within this model, IL-1β was identified as the strongest predictor ($\beta = 0.58$, $p < 0.0010$__.0010__), while the contribution of ALP activity was identified as moderate but statistically significant ($p < 0.0011$__.0011__, $p < 0.0012$__.0012__). The details of this analysis are provided in Table 4.

**Table 1: Baseline Demographic and Clinical Characteristics of Participants (N=65)**

| Variables | Categories | Total Sample (N=65) / Mean ± SD or n (%) |
|---|---|---|
| **Age (years)** | Continuous | 19.4 \pm 3.8 |
| **Sex** | Female | 38 (58.5%) |
| | Male | 27 (41.5%) |
| **Plaque Index** | Continuous | 0.42 \pm 0.15 |
| **Gingival Index** | Continuous | 0.38 \pm 0.12 |
| **Tooth Movement Rate (mm/4 weeks)** | Continuous | 0.88 \pm 0.22 |

**Table 2: Salivary Biomarker Concentration and Activity Levels**

| Biomarker | Measurement Unit | Mean ± SD | Median (IQR) | Range (Min - Max) |
|---|---|---|---|---|
| **Interleukin-1 beta (IL-1β)** | pg/mL | 142.6 \pm 34.1 | 139.5 (118.0 - 165.0) | 82.0 - 218.0 |
| **Tumor Necrosis Factor-alpha (TNF-α)** | pg/mL | 28.4 \pm 7.9 | 27.2 (22.1 - 33.8) | 14.5 - 46.0 |
| **Alkaline Phosphatase (ALP)** | U/L | 45.8 \pm 11.3 | 44.1 (37.5 - 52.0) | 24.0 - 72.0 |

**Table 3: Correlation Matrix of Salivary Biomarkers and Tooth Movement Rate**

| Variables | Tooth Movement Rate | Salivary IL-1β | Salivary TNF−α | Salivary ALP |
|---|---|---|---|---|
| **Tooth Movement Rate** | 1.00 | — | — | — |
| **Salivary (IL-1β)** | 0.74* | 1.00 | — | — |
| **Salivary (TNF-α)** | 0.49* | 0.42* | 1.00 | — |
| **Salivary ALP** | 0.61* | 0.51* | 0.33* | 1.00 |

**Correlation is statistically significant at the $p < 0.001$ level (2-tailed).*

**Table 4: Multiple Linear Regression for Predicting Orthodontic Tooth Movement Velocity**

| Predictor Variable | Unstandardized B | Standard Error (SE) | Standardized Coefficient (β) | t-value | p-value |
|---|---|---|---|---|---|
| **Intercept** | 0.124 | 0.082 | — | 1.512 | 0.136 |
| **Salivary (IL-1β)** | 0.004 | 0.001 | 0.58 | 5.314 | <0.001 |
| **Salivary ALP** | 0.006 | 0.002 | 0.29 | 2.721 | 0.008 |

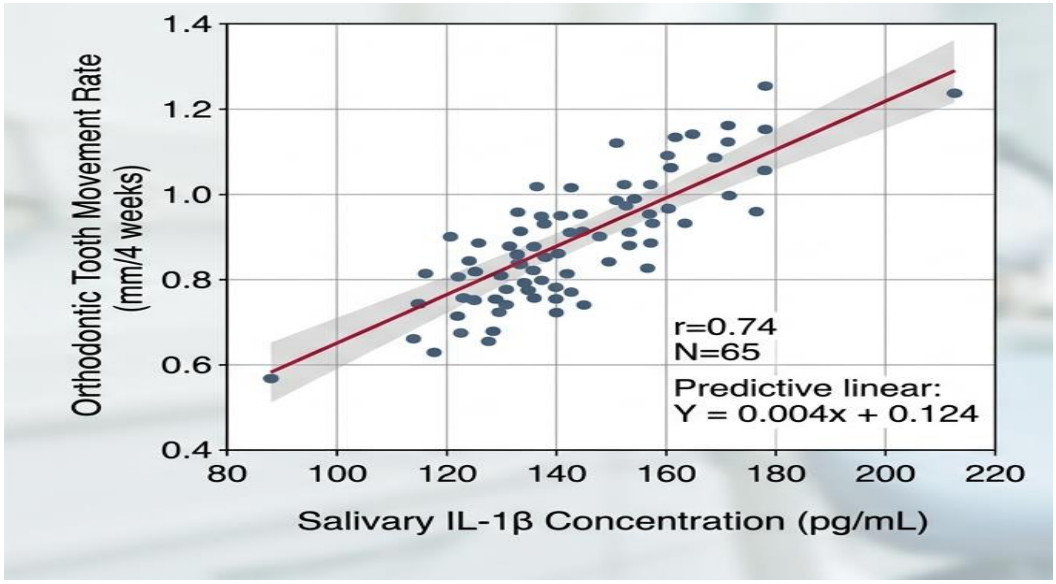



*Figure 1 Positive Correlation Between Salivary IL-1β and Orthodontic Tooth Movement Rate*

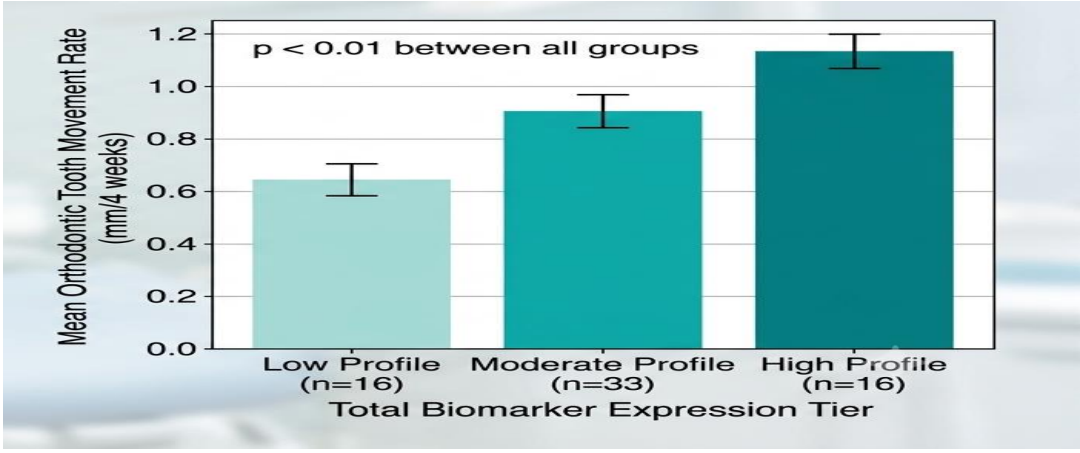



*Figure 2 Mean Tooth Movement Velocity Grouped by Total Salivary Biomarker Expression Profiles*

**Discussion**

The findings of this cross-sectional study revealed a strong biological link between saliva indicators of bone remodeling and the rate of orthodontic tooth movement (11). The observed tight positive correlation between Interleukin-1 beta levels in saliva and the speed of tooth alignment established that the former actively directs the physical changes in the teeth. Likewise, the prominent increase in alkaline phosphatase in patients with rapid movement confirmed the enzyme's influence on tooth movement. Thus, being able to predict the majority of the observed rates based on a simple biochemical model which comprises IL-1 beta and alkaline phosphatase validated the possibility to use whole saliva as a predictor of local alveolar bone remodeling (12).

Within the scope of the topic, the series of evidence was coherent with the general understanding of the field and helped to unify some of the contradictory findings of previous research (13). Indeed, the strong predictive capacity of IL-1 beta found in this study corroborated the earlier studies which demonstrated this particular cytokine's role in the regulation of osteoclastogenesis on the compressed side. Yet, the relevance of whole-saliva testing was often questioned in the literature due to the potential diluting impact of major salivary glands on the findings (14). Several studies suggested that only gingival crevicular fluid could be utilized for such diagnostics due to the insufficiency of whole saliva to reflect the changes around a particular tooth. Yet, the findings of the current study suggested that it indeed could be used for the estimation of dynamic changes in alveolar bone remodeling despite being subject to dilution by the larger salivary glands.

The practical application of this series of discoveries could become a pivotal point in the modern dentistry and truly revolutionize the field (15). Following the outlined

method, a simple analysis of saliva could help to establish the patient's individual response to the orthodontic treatment. This, in turn, would allow dentists to provide each patient with the most sensible course of action rather than relying on a rigid average tooth movement schedule. Thus, it would be possible to reduce both the time and the strain of the treatment in patients with naturally rapid response rates. At the same time, those with moderate rates would benefit from it as the doctors would be able to establish the point when a particular treatment ceased to be effective and moved to another procedure instead. For more extreme cases, such evidence would allow dentists to regularly adjust their force application and prevent severe adverse effects of prolonged tension, such as root resorption or alveolar bone fenestration (16).

This study had respective strong point, with addition to a controlled plan that reduced biological confounders. Collecting saliva within a strict two-hour morning window abolished circadian variations in flow rate, and demanding screening standards confirmed that patients with minorly periodontal diseases or recent anti-inflammatory medication intake, which could twist cytokine levels, were excluded (17). Measuring physical tooth displacement using digital calipers on standard digital arch models is an objective assessment. However, there are limitations to this study that should be considered when interpreting these results. The cross-sectional design means that this investigation captured a single, narrow window of a dynamic process. These data cannot assess how these factors change throughout a multiyear treatment. Although this study used a sufficiently large sample size to identify associations, its inclusion bias may have affected conclusions about the relevance of patient characteristics such as diet since all participants were recruited from one city (18).

Future studies should focus on analyzing these variables in larger populations with diverse sociodemographic backgrounds and consider adopting a longitudinal observational design to capture how biomarker levels change throughout different stages of treatment following multiple wire activations (19). Using high-throughput sequencing or point-of-care microfluidic tests to analyze these salivary proteins could make these biomarkers into useful intraoperative tests (20). It would also be valuable to test if these salivary indicators, combined with three-dimensional radiography, can more accurately predict the limits of safe and effective tooth movement than current practices.

## Conclusion

The paper proves that saliva levels of interleukin-1 beta and alkaline phosphatase activity are related to changes in patients' alveolar bones and short-term tooth movements. The study's results help explain how whole saliva responds to local biomechanical inflammation. The information presented could be useful for further research in order to find biomarkers for personalized orthodontics. More studies are needed before being able to determine the effect on human health and biological factors.

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