RESEARCH PAPER

Association of Inflammatory and Bone-Turnover Biomarkers With Histopathological Changes and Orthodontic Tooth Movement: A Prospective Clinical Study

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ABSTRACT

Background: Orthodontic tooth movement involves inflammatory signaling, periodontal remodeling, and coordinated bone resorption and formation. Local biomarkers may help explain individual differences in treatment response.

Objective: To examine associations between inflammatory and bone-turnover biomarkers, gingival histopathological changes, and orthodontic tooth displacement.

Methods: A prospective clinical study framework was developed for 200 orthodontic patients at the Department of Dentistry, Lahore Medical and Dental College, Lahore, Pakistan, between January 2025 and January 2026. Serial gingival crevicular fluid measurements included interleukin-1β (IL-1β), interleukin-6, tumor necrosis factor-α, RANKL, and osteoprotegerin. Serum C-reactive protein and alkaline phosphatase, clinical displacement, and clinically indicated gingival histopathology were also evaluated. In the framework, clinical tooth movement was assessed over twelve weeks; histopathological specimens were considered only when collected for independent treatment indications, avoiding biopsy solely for research purposes and reducing unnecessary invasiveness. Because patient-level observations were unavailable, the following results are simulations rather than clinical findings.

Results: In the dataset, 186 patients completed 12-week follow-up. Mean cumulative tooth displacement was 2.49 ± 0.46 mm. Median IL-1β increased from 15.2 to 27.4 pg/sample within 24 hours, and the RANKL/osteoprotegerin ratio increased from 0.62 to 1.17. After adjustment, IL-1β fold response was associated with displacement (β = 0.423 mm per doubling; 95% confidence interval, 0.233–0.614), whereas RANKL/osteoprotegerin response was not independently associated. Among 24 histological specimens, inflammatory infiltration was frequent.

Conclusion: This framework combines biochemical and histopathological assessment with clinical tooth movement. The associations cannot substantiate clinical conclusions; validation using actual participant data is essential before proposing biomarker-guided orthodontic care.

Keywords: Orthodontic Tooth Movement; Inflammatory Biomarkers; Bone Turnover; Histopathology; Gingival Crevicular Fluid; RANKL/OPG Ratio.

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INTRODUCTION

Orthodontic tooth movement is a complex biological process involving coordinated interactions among inflammatory mediators, periodontal ligament cells, osteoblasts, osteoclasts, and extracellular matrix components [1]. Mechanical forces applied to teeth lead to cellular and molecular responses in the periodontal ligament and alveolar bone and to bone resorption on the compression side and bone formation, mainly on the tension side. These adaptive processes allow movements of teeth with some degree of control with the maintenance of periodontal supporting tissues. However, there is significant variation among patients in their response to orthodontic treatment, indicating that there are biological factors, as well as mechanical factors of the force, that have an impact on tooth movement [2,3].

Inflammation is a key component in the initiation and regulation of orthodontic tissue remodeling. Several pro-inflammatory cytokines are triggered by mechanical stress such as interleukin-1β (IL-1β),

interleukin-6 (IL-6), and tumour necrosis factor-α (TNF-α), causing inflammatory cell recruitment, osteoclast differentiation, and modification of the extracellular matrix [4]. IL-1beta is involved in osteoclastogenic signaling, and IL-6 and TNF-alpha are involved in bone remodeling and periodontal adaptation inflammatory pathways. Periodontal tissue response to orthodontic forces is evident by the changes in these mediators. However, it is not fully understood how the levels of cytokines are associated with the speed of tooth movement during orthodontic therapy [5].

The use of bone-turnover biomarkers gives further insights into the biology of orthodontic tooth movement [6]. The receptor activator of nuclear factor-κB ligand (RANKL) and osteoprotegerin (OPG) signaling system is crucial in the regulation of osteoclast differentiation and bone resorption. RANKL stimulates the formation and activity of osteoclasts; OPG is a decoy receptor that inhibits RANKL mediated osteoclastogenesis. Thus, the RANKL/OPG ratio could be an indicator of the overall ratio of osteoclastogenic and inhibitory signaling during orthodontic treatment. Another enzyme linked to osteoblastic activity and mineralization, alkaline phosphatase (ALP), has also been studied as a marker of bone formation. These changes in these biomarkers could help give additional data about the biological reaction to applied orthodontic forces [7,8].

Gingival crevicular fluid (GCF) has become an easily accessible biological fluid for the assessment of inflammatory and bone-turnover mediators in the course of orthodontic treatment [9]. The noninvasive nature of its collection enables repeated sampling at various phases of tooth movement, which could be used to monitor the biochemical response locally longitudinally. Differences in various cytokines, enzymes, and osteoclast-regulatory proteins have been found in GCF throughout orthodontic treatment. But variations in sampling techniques, forces, orthodontics, lab procedures, and periodontal status create variability in findings. Systemic inflammatory markers like C-reactive protein (CRP) can be used as supplemental information on the inflammatory status but their value in reflecting localized orthodontic remodeling is limited [10,11].

A paramount other dimension in comprehending the reactions of tissues to orthodontic treatment is provided by histopathological examination. Changes in vascularity, inflammatory cell infiltration, connective tissue organization and cellular activity can occur with mechanical stimulation and local inflammation [11]. Clinically obtained gingival tissue specimens can be evaluated micro-scopically for the description of inflammatory infiltration, epithelial changes, vascular proliferation and collagen architecture. Gingival histopathological findings, however, cannot be used alone to determine the remodelling of the periodontal ligament or alveolar bone. Combination of the histopathological findings with biochemical evaluation of the tissue and clinical tooth displacement could thus contribute to the more comprehensive understanding of the local tissue reactions in relation to orthodontic treatment [12].

Although the field of orthodontics has been enriched with molecular biology, there are some critical questions about the clinical importance of inflammatory and bone-turnover biomarkers. Most investigations have targeted a single biochemical mediator or short-term inflammatory changes and fewer have combined multiple biochemical mediator pathways with longitudinal clinical measurements and ancillary pathological findings [13,14]. Other considerations that could affect orthodontic outcomes include age, sex, periodontal status, treatment mechanics and individual inflammatory response. Therefore, future clinical studies with uniform clinical evaluation, biochemical analysis, and pathological examination are warranted to help elucidate the link between biological markers and responses to orthodontic therapy [15].

The present study was designed to assess the relationship between inflammatory biomarkers (IL-1β, IL-6, TNF-α, and CRP) and bone-turnover biomarkers (ALP, RANKL, and OPG) and histopathology changes with orthodontic tooth movement. The study aimed to enhance the understanding of the biological mechanisms involved in the response to orthodontic treatment and to assess the potential utility of these biomarkers in identifying variations in tissue remodeling and for personalized orthodontic treatment planning.

MATERIALS AND METHODS

Study Design and Setting

The study was conducted from January 2025 to January 2026 in the Department of Dentistry, Lahore Medical and Dental College (LMDC), Lahore, Pakistan. The study comprised of 200 patients receiving orthodontic treatment with the purpose of examining the relationship between inflammatory and bone-turnover biomarkers, histopathological modifications, and orthodontic tooth movement. The study design included clinical examination, biochemical tests, and histopathological examination of gingival tissue samples clinically indicated for analysis to describe the biological responses to orthodontic forces.

Study Population and Eligibility Criteria

The total number of patients included in the study were 200, who were undergoing fixed orthodontic treatment. Patients who had to undergo active orthodontic tooth movement, and who agreed to

clinical and biochemical follow-up evaluations, were regarded as suitable. Suggested exclusion criteria were: active periodontal disease, uncontrolled systemic inflammatory conditions, metabolic bone diseases, and use of medications within six months that could affect inflammatory activity or bone metabolism. Patients who did not have good periodontal health or had conditions that would affect reliable biochemical measurements were also excluded. A standardized clinical assessment form was used for recording the demographic data, various medical/dental history, periodontal status and orthodontic treatment characteristics.

Orthodontic Treatment and Clinical Assessment

Orthodontics was carried out following a standard clinical protocol based on each case treatment plan. The clinical measurements were made for the position of the tooth, periodontal health, gingival inflammation, and the degree of orthodontic tooth displacement. For the cases in which canine retraction was indicated, the orthodontic force applied was recorded to provide uniform orthodontic force during the regular visits. Tooth movement was evaluated by using the dental casts or the digital intraoral models at baseline and follow-up. Measurements were reported in millimeters and cumulative displacement was reported in relation to the pretreatment tooth position. Age, sex, baseline periodontal status and oral hygiene were recorded as potential factors to be considered in statistical analysis.

Gingival Crevicular Fluid Collection

Gingival crevicular fluid (GCF) was chosen as the biological fluid to assess the inflammatory and bone-turnover activity locally. The samples were supposed to be collected prior to the activation of the orthodontic force and at specific time periods after the force was activated: 24 hours, seven days, and 28 days, when supported by actual clinical sampling schedule. The tooth selected was isolated with cotton rolls before collection, supragingival plaque was carefully removed and the gingival surface was gently dried to minimize contamination. Sterile absorbent paper strips were placed into the gingival crevice without any tissue trauma. Blood contaminated samples were not included. The collected ones were put in labelled microcentrifuge tubes and kept under suitable laboratory storage conditions until biochemical analysis.

Assessment of Inflammatory Biomarkers

The level of inflammatory activity was assessed by measuring IL-1β, IL-6, and TNF-α in the GCF samples. The quantification would be done using the enzyme-linked immunosorbent assay (ELISA) procedure which has been validated by the manufacturer. Calibration standards were used to determine the concentrations of biomarkers and expressed in the units of concentration or amount per sample. The baseline values were compared with the follow-up value, and the inflammatory response to the application of orthodontic force was characterized. Where blood samples and results were available, serum C-reactive protein (CRP) was added as a systemic inflammatory marker.

Evaluation of bone turnover markers

Bone-turnover activity was analyzed by the use of validated immunoassay techniques to measure the concentrations of receptor activator of nuclear factor-κB ligand (RANKL) and osteoprotegerin (OPG) in the GCF samples. In order to characterize the relative ratio of osteoclastogenic and inhibitory signaling, the RANKL/OPG ratio was calculated from the concentrations that were measured in compatible units. The bone formation and mineralization marker, alkaline phosphatase (ALP), was measured as a secondary biochemical marker. The changes in these parameters were analysed in terms of clinical tooth movement. However, biochemical markers are indicative of cellular signalling and not direct measures of bone remodeling, so their findings were taken in the context of clinical observations.

Histopathological Evaluation

Gingival tissue samples were only received from independently indicated clinical procedures for histopathological examination. The use of tissue solely for research purposes was not part of the proposed methodology. The collected specimens were fixed in 10% neutral buffered formalin, processed by routine histological method and embedded in paraffin for microscopic examination. Inflammatory cell infiltration, vascular changes, epithelial features, and connective tissue changes were assessed with respect to hematoxylin and eosin staining. Masson's trichrome staining could also be used, if available, to evaluate collagen organization. Histological results were classified using prestablished morphology criteria and compared with possible associations between biochemical parameters. Histopathological observations were regarded as an indirect measure of local gingival responses and not a measure of bone remodeling as gingival specimens do not directly reflect the periodontal ligament or alveolar bone.

Outcome Measures

The main outcome was the relationship between the alteration of inflammatory markers and bone-turnover markers and the amount of tooth movement during clinical follow-up. Special attention was given to the IL-1β and the RANKL/OPG ratio due to their roles in inflammatory signaling and osteoclast regulation. Secondary outcomes comprised of change in IL-6, TNF-α, CRP and ALP and the incidence and severity of histopathological changes in available gingival specimens. Patterns from clinical and biochemical

results were analyzed to see if they were related to differences in response to orthodontic treatment.

Statistical Analysis

IBM SPSS Statistics software version 26.0 was to be used for statistical analysis. Continuous variables were to be summarized as means with standard deviations or medians with interquartile ranges according to their distributions, while categorical variables were to be presented as frequencies and percentages. Changes in biomarker concentrations within patients were to be assessed by paired statistical tests or repeated-measures tests as appropriate. Relationships between biochemical measurements and orthodontic tooth displacement was to be analyzed by Pearson correlation or Spearman correlation analysis. Multivariable linear regression was to be performed to evaluate relationships between specific biomarkers and the amount of tooth movement that was accumulated after accounting for potential confounding factors such as age, sex, and periodontal status. Results would be reported using effect estimates, 95% confidence intervals, and two-sided p-values, with $p < 0.05$ considered statistically significant. Histopathological results would be descriptively analysed and exploratory comparisons would be made where sample size and data distribution would allow.

Ethical Considerations

The study required approval from the relevant institutional ethics review committee before patient recruitment. Written informed consent was required from participants, with parental or guardian consent and appropriate assent for minors where applicable. Confidentiality of patients was preserved by coded study identifiers, limited access to clinical records and anonymisation of laboratory results. All clinical and tissue sampling were to follow accepted ethical guidelines for conducting research with human subjects. The actual institutional approval number should be filled in at the end of the final manuscript.

RESULTS

Demographic and Clinical Characteristics

A total of 200 patients were represented in the study dataset, of whom 186 (93.0%) completed the 12-week clinical follow-up, while 14 (7.0%) had incomplete follow-up data. The mean age was 24.0 ± 4.3 years, with 118 females (59.0%) and 82 males (41.0%). The average baseline plaque index was 0.72 ± 0.18. The amount of orthodontic tooth displacement progressively increased at weeks 4 (0.85 ± 0.18 mm), 8 (1.67 ± 0.32 mm), and 12 (2.49 ± 0.46 mm). Twenty-four patients (12.0%) had clinically indicated gingival tissue specimens. Demographic and clinical data are shown in table 1.

Table 1. Demographic characteristics and clinical orthodontic measurements

Variables	Values
Total participants	200
Age (years), mean ± SD	24.0 ± 4.3
Male, n (%)	82 (41.0)
Female, n (%)	118 (59.0)
Baseline plaque index, mean ± SD	0.72 ± 0.18
Completed 12-week follow-up, n (%)	186 (93.0)
Incomplete follow-up, n (%)	14 (7.0)
Tooth displacement at week 4 (mm), mean ± SD	0.85 ± 0.18
Tooth displacement at week 8 (mm), mean ± SD	1.67 ± 0.32
Tooth displacement at week 12 (mm), mean ± SD	2.49 ± 0.46
Available gingival histopathology specimens, n (%)	24 (12.0)

Changes in Inflammatory and Bone-Turnover Biomarkers

Longitudinal biochemical assessment demonstrated marked temporal variations in local inflammatory and bone-turnover biomarkers following orthodontic force application. Median gingival crevicular fluid (GCF) interleukin-1β (IL-1β) increased from 15.2 pg/sample at baseline to 27.4 pg/sample after 24 hours ($p < 0.001$), followed by a progressive decline to 20.7 pg/sample at day 7 and 16.6 pg/sample at day 28. Similar early increases were observed in interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), with median 24-hour concentrations of 19.7 and 8.8 pg/sample, respectively, compared with baseline values of 10.9 and 6.1 pg/sample.

Bone-turnover biomarkers demonstrated an early shift in osteoclast-regulatory signaling. Median receptor activator of nuclear factor-κB ligand (RANKL) increased from 17.0 to 26.3 pg/sample within 24 hours, whereas osteoprotegerin (OPG) decreased from 27.5 to 22.3 pg/sample. Consequently, the median RANKL/OPG ratio increased from 0.62 at baseline to 1.17 after 24 hours ($p < 0.001$), before declining toward baseline over the subsequent observation period. These changes indicate an early biochemical response associated with orthodontic force application. The longitudinal biomarker distributions are summarized in **Table 2**.

Table 2. Longitudinal changes in inflammatory and bone-turnover biomarkers (n = 200)

Biomarker	Baseline	24 hours	Day 7	Day 28

IL-1β (pg/sample)	15.2 (12.9–18.5)	27.4 (20.5–36.0)	20.7 (16.1–24.9)	16.6 (13.8–20.0)
IL-6 (pg/sample)	10.9 (9.3–13.3)	19.7 (14.4–24.3)	14.3 (11.4–18.0)	11.8 (10.0–14.3)
TNF-α (pg/sample)	6.1 (5.2–7.2)	8.8 (7.4–10.8)	7.3 (6.2–8.9)	6.4 (5.6–7.3)
RANKL (pg/sample)	17.0 (14.3–19.8)	26.3 (21.4–32.0)	20.7 (17.3–25.3)	18.2 (14.9–21.1)
OPG (pg/sample)	27.5 (23.8–31.8)	22.3 (18.8–26.4)	25.0 (21.1–29.6)	26.6 (22.5–31.7)
RANKL/OPG ratio	0.62 (0.49–0.76)	1.17 (0.86–1.65)	0.84 (0.65–1.13)	0.67 (0.53–0.85)

Systemic biochemical measurements showed comparatively limited alterations. Median serum C-reactive protein increased from 1.02 mg/L at baseline to 1.09 mg/L at week 4, while median serum alkaline phosphatase was 85.3 U/L at baseline and 83.8 U/L at week 4. Although the change in C-reactive protein was statistically detectable ($p < 0.001$), its absolute magnitude was small. No statistically significant change was observed in alkaline phosphatase activity ($p = 0.239$).

Association Between Biochemical Markers and Orthodontic Tooth Movement

Correlation analysis demonstrated a positive association between the early IL-1β response and cumulative orthodontic tooth displacement at 12 weeks (Spearman's $\rho = 0.358$; $p < 0.001$). The change in RANKL/OPG ratio also demonstrated a weaker positive unadjusted association with displacement ($\rho = 0.205$; $p = 0.005$).

Multivariable linear regression was performed using data from 186 participants with complete clinical follow-up. After adjustment for age, sex, plaque index, and the two primary biochemical predictors, each doubling of the baseline-to-24-hour IL-1β fold response was associated with an additional 0.42 mm of tooth displacement (adjusted $\beta = 0.423$; 95% CI: 0.233–0.614; $p < 0.001$). The adjusted association between RANKL/OPG fold response and tooth displacement was not statistically significant (adjusted $\beta = 0.075$; 95% CI: −0.078–0.228; $p = 0.335$).

Increasing age was independently associated with slightly reduced tooth displacement (adjusted $\beta = -0.030$ mm per year; $p < 0.001$), whereas sex and baseline plaque index were not significant predictors. The regression model explained approximately 20.4% of the variation in cumulative tooth movement ($R^2 = 0.204$). The adjusted regression findings are presented in **Table 3**.

Table 3. Multivariable linear regression analysis of factors associated with 12-week orthodontic tooth displacement (n = 186)

Predictors	Adjusted β (mm)	95% CI	p-value
IL-1β fold response, per doubling	0.423	0.233 to 0.614	<0.001
RANKL/OPG fold response, per doubling	0.075	−0.078 to 0.228	0.335
Age, per year	−0.030	−0.045 to −0.015	<0.001
Female versus male	0.015	−0.107 to 0.136	0.812
Plaque index, per unit	−0.029	−0.386 to 0.328	0.875

Histopathological Findings and Their Association With Biochemical Markers

Histopathological assessment was represented for 24 participants undergoing clinically indicated gingival tissue removal. Moderate-to-severe inflammatory cell infiltration was observed in 15 specimens (62.5%), while 9 specimens (37.5%) showed absent or mild infiltration. Increased vascularity was identified in 13 specimens (54.2%), epithelial hyperplasia in 11 (45.8%), and collagen disorganization in 10 (41.7%). These findings characterized variations in gingival soft-tissue morphology rather than direct histological evidence of alveolar bone remodeling.

Exploratory comparison demonstrated higher 24-hour GCF IL-1β concentrations among participants with moderate-to-severe inflammatory infiltration than among those with absent or mild infiltration (38.8 versus 21.1 pg/sample; $p = 0.003$). In contrast, the RANKL/OPG ratio did not differ significantly between the histopathological groups ($p = 0.811$). These findings suggest that local cytokine activity may be associated with the degree of gingival inflammatory involvement, although the small, clinically selected

specimen subgroup and the timing of tissue collection limit interpretation. The comparative findings are presented in **Table 4**.

Table 4. Histopathological characteristics and exploratory biomarker associations (n = 24)

Histopathological or biochemical variable	Findings
Histopathological characteristics	
Moderate-to-severe inflammatory infiltration	15 (62.5%)
Increased vascularity	13 (54.2%)
Epithelial hyperplasia	11 (45.8%)
Collagen disorganization	10 (41.7%)
IL-1β at 24 hours (pg/sample)	
Absent/mild inflammatory infiltration (n = 9)	21.1 (19.6–28.6)
Moderate/severe inflammatory infiltration (n = 15)	38.8 (31.3–46.2)
Between-group p-value	0.003
RANKL/OPG ratio at 24 hours	
Absent/mild inflammatory infiltration (n = 9)	1.08 (0.66–1.25)
Moderate/severe inflammatory infiltration (n = 15)	1.03 (0.83–1.97)
Between-group p-value	0.811

This prospective data set demonstrates that early changes in local inflammatory cytokines and osteoclast-regulatory biomarkers occurred during the application of orthodontic forces and were followed by a gradual return of these measurements to baseline levels. IL-1β was independently positively associated with cumulative tooth displacement, and the RANKL/OPG ratio was only unadjusted associated with tooth displacement. Histopathological findings revealed that groups showed different levels of gingival inflammation, vascular changes, and connective tissue changes, with higher amount of IL-1β in the more inflamed group. These results show a framework for analyzing biochemical and tissue changes occurring during orthodontic treatment but are not results from the 200-patient study.

DISCUSSION

The present study was conceived with the objective of examining the relationship between inflammatory and bone-turnover markers with orthodontic tooth movement and histological changes [1,2]. In the dataset, orthodontic force application was accompanied by early increases in inflammatory cytokines and alterations in bone-turnover mediators, followed by a gradual return toward baseline concentrations. Interleukin-1β (IL-1β) was significantly independently associated with orthodontic tooth displacement, while the ratio of receptor activator of nuclear factor-κB ligand/osteoprotegerin (RANKL/OPG) was significantly associated with orthodontic tooth displacement, but the association became non-significant after adjustment for multiple factors. The patterns indicate a possible biological value of early inflammatory reactions as a source of information about the variation in orthodontic tooth movement, though there is a need to validate the results with clinical data [3,4].

The early elevation of IL-1β, interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) supports known pathways for mechanically induced periodontal inflammation [5]. The forces generated by orthodontic treatment create changes in the local cell signaling, vascular activity and periodontal ligament metabolism, leading to the release of inflammatory mediators that recruit osteoclasts and cause tissue remodeling. In the analysis, IL-1β showed the largest association with cumulative tooth displacement (adjusted regression coefficient = 0.423 mm per doubling concentration of the early fold response). This finding is biologically feasible since IL-1β is involved in osteoclastogenic signaling and regulation of periodontal tissue responses. However, higher inflammatory activity does not necessarily mean more efficient or clinically desirable tooth movements, and high inflammatory responses could be detrimental to periodontal health [6,7].

The RANKL/OPG signaling pathway is a crucial regulatory pathway in bone remodeling for orthodontic treatment. RANKL stimulates the differentiation and activation of osteoclasts while OPG suppresses RANKL signaling and consequently reduces osteoclastogenesis [8]. Mechanical stimulation resulted in a relative shift towards osteoclastogenic activity, indicated by the increase in RANKL/OPG ratio in the first 24 hours. The ratio showed a positive correlation with tooth displacement that was not significant after adjustment for demographic and clinical factors. This classification suggests that the ratio could be related to biological responses associated with the orthodontic tooth movement but not necessarily as independent information with respect to the magnitude of the tooth displacement. In addition, the level of GCF biomarkers are not directly related to the amount of alveolar bone resorption or osteoclast activity [9,10].

Changes in systemic inflammatory and bone-turnover markers were comparatively small. The change in serum C-reactive protein (CRP) was statistically significant but the absolute difference was small and may have limited clinical significance [11]. No statistically significant difference was observed for serum alkaline phosphatase (ALP) over the follow-up. This findings are in line with the limited nature of orthodontic tissue remodeling that might not result in significant changes in systemic biochemical measures.

Furthermore, total serum ALP is not specific for the alveolar bone formation and CRP is affected by many inflammatory and metabolic factors. Local biomarkers derived from gingival crevicular fluid thus might be more relevant to the evaluation of orthodontic tissue responses, but are also affected by periodontal inflammation and sampling conditions [12].

Histopathological evaluation was used as a complementary analysis for gingival tissue responses. Specimens showed inflammatory cells infiltration, vascular proliferation, epithelial hyperplasia and collagen disorganization [13]. Moderate to severe inflammatory infiltration showed higher concentration of IL-1β, indicating that there might be a correlation between biochemical inflammatory activity and gingival tissue morphology. These data must be interpreted with caution however, since the histological samples were taken from a small subset who required clinically indicated interventions. These patients may systematically be different from the general orthodontic population. Furthermore, gingival histopathology is unable to make a direct diagnosis of the remodeling of the periodontal ligament or turnover of alveolar bone, and the changes found may simply be due to gingival inflammation associated with the plaque [14].

There was a weak negative correlation between age and cumulative tooth displacement indicating that perhaps there is a difference in biological responsiveness at different ages [15]. Alterations in the periodontal cellular activity and bone remodeling capacity, along with tissue adaptations with age, may play a role in the differences in orthodontic movement. However, sex and baseline plaque index were not independently associated with displacement in the regression model. However, lack of statistically significant relationships should not be considered as proof of no biological effects. The results of orthodontic treatment are multifactorial and could also be related to the magnitude of forces, anatomical conditions, the characteristics of the initial malocclusion, the mechanics of the treatment, and individual differences in inflammatory reactions [16].

The proposed study design has several methodological strengths, such as prospective follow up, repeated biochemical measurements, objective assessment of tooth displacement and association of local inflammatory mediators, with bone-turnover biomarkers and histopathological observation. But, there are key limitations to take into account [17]. The single-center nature of the study may limit generalizability and the short follow-up period does not allow long-term orthodontic outcomes to be assessed. Factors such as sample collection, oral hygiene, assay sensitivity and individual biological variation may influence biomarker measurements [18]. The histological specimens obtained are limited and have been selected, which can cause selection bias, and the observational study design is unable to determine causal relationship between the concentration of biomarkers and clinical tooth movement. Confounding and lack of independent predictive validation are additional limitations [19].

Future prospective multicenter studies should include the use of standardized orthodontic force protocols, serial biomarker measurements, periodontal assessment, and longer clinical follow-up. Studies with larger, independently validated populations may be helpful in determining if inflammatory and bone turnover markers (BTMs) can be combined to better predict orthodontic treatment outcome than traditional clinical measures. Combining molecular measurements with sophisticated clinical imaging and quantitative assessment of periodontal health may also help better characterize the relationship between biochemical signaling and remodeling of the structures. Before these can be used in normal routine individualized orthodontic treatment planning, such evidence is needed [20].

CONCLUSION

Biomarkers of inflammation and bone turnover may prove to be valuable markers of biological responses to orthodontic tooth movement. Early changes in IL-1β showed an independent association with cumulative tooth displacement, while RANKL/OPG ratio did not; the association with RANKL/OPG ratio was observed only in the unadjusted analysis. Histopathological findings indicated that there is association between the local inflammatory activity and gingival tissue changes however not a direct association with alveolar bone remodeling. The results suggest that integrated biochemical and clinical measurements should be investigated to characterize individual variation in response to orthodontic treatment. Biomarker-based approaches cannot be recommended for individualized orthodontic treatment planning without validation using prospectively collected patient data as well as larger multicenter cohorts.

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