Natural Resourcesfor Human Health
eISSN: 2583-1194

Original Research

Received 25 June 2026
Revised 15 Aug 2026
Accepted 05 Sep 2026

Natural Resources for Human Health 2026, Vol. 6 (10s): 194–200

KEYWORDS:
Orthodontic tooth movement; Gingival crevicular fluid; RANKL; OPG; Bone metabolism; Maxilla; Mandible.

To Compare Bone Metabolism in Maxilla and Mandible using Biochemical Markers in Gingival Crevicular Fluid During Early Orthodontic Tooth Movement

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ABSTRACT:

BACKGROUND: Orthodontic tooth movement involves bone remodeling, which can be assessed through biochemical changes in gingival crevicular fluid (GCF). RANKL and OPG are important markers involved in bone resorption and formation.

Aim: To compare bone metabolism in the maxilla and mandible using RANKL and OPG levels in GCF during early orthodontic tooth movement.

MATERIALS AND METHODS: GCF samples were collected from the maxillary and mandibular regions at baseline, 15 days, and 1 month after orthodontic force application. RANKL and OPG levels were assessed, and the RANKL/OPG ratio was calculated. The data were analysed using independent t-test, repeated-measures ANOVA and Bonferroni post-hoc test.

RESULTS: RANKL levels showed a significant increase following orthodontic force application, with higher mandibular levels particularly in males. OPG levels showed variations between the maxilla and mandible at different time points. The RANKL/OPG ratio was highest in the male mandible at 15 days (1.56), suggesting greater bone resorption during the early phase of tooth movement.

CONCLUSION: Bone metabolism showed differences between the maxilla and mandible during early orthodontic tooth movement. The mandible, particularly in males, showed a more pronounced early bone-resorptive response.

INTRODUCTION

Orthodontic tooth movement is a biological process involving coordinated changes in the periodontal ligament and alveolar bone, with bone resorption and formation occurring in response to orthodontic force.[14–20]

Natural Resourcesfor Human Health
eISSN: 2583-1194

The RANK–RANKL–OPG system plays an important role in bone remodeling. RANKL promotes osteoclast differentiation and bone resorption, while OPG regulates this process by inhibiting RANKL activity.[7,9] Mechanical orthodontic force can alter RANKL and OPG levels, particularly during the early phase of tooth movement.[10,13]

Gingival crevicular fluid (GCF) is a minimally invasive medium that can reflect biochemical changes occurring in periodontal tissues during orthodontic tooth movement. Studies have demonstrated changes in RANKL, OPG and other biomarkers in GCF following orthodontic force application.[12,15] Recent studies have also supported the use of GCF for evaluating biological changes during orthodontic treatment.[3,6]

Although several studies have evaluated biochemical changes during orthodontic tooth movement, comparative information regarding bone metabolism between the maxilla and mandible during the early phase is limited. Hence, the present study was undertaken to compare bone metabolism in the maxilla and mandible using RANKL and OPG levels in GCF during early orthodontic tooth movement.

OBJECTIVES OF THE STUDY:

- To assess the expression of receptor activator of nuclear factor kappa B ligand, and osteoprotegerin in the gingival crevicular fluid of teeth undergoing orthodontic forces.
- To compare the biomarker differences in maxilla and mandible .

METHOD OF COLLECTING DATA:

- For every patient, samples will be taken from both maxillary and mandibular arches , Each sample will be utilized to identify two distinct biomarkers.
- Samples will be collected at 3 time intervals :

a. 1st sample collected at baseline before commencement of fixed orthodontic treatment.

b. 2nd sample collected after 15 days after the commencement of fixed orthodontic treatment.

c. 3rd sample collected after 1 month after the commencement of fixed orthodontic treatment.

INCLUSION CRITERIA

- Overall good health.
- Patient willing to undergo fixed orthodontic treatment.
- Patient with Angle's class I malocclusion with mild crowding, spacing and bimaxillary protrusion.
- Teeth from 1-7 should be present in all the quadrants.

EXCLUSION CRITERIA

- Smokers.
- Gingivitis.
- Probing pocket depths of ≥4 mm , clinical attachment loss of ≥2 mm in the chosen or adjacent teeth.
- Use of antibiotics or anti-inflammatory drugs in the six months prior.

PROCEDURE:

Figure no 1: Armamentarium for GCF collection

The selected test site was air-dried and isolated with sterile cotton rolls. Supragingival plaque was removed gently without touching the marginal gingiva to avoid bleeding from gingiva. By using an extracrevicular (unstimulated) method, a standard volume of GCF was collected from each selected site.

Figure no 2: Samples being collected using microcapillary pipette

The GCF samples obtained was wrapped in foil and then quickly moved to a centrifuge tube that had screw caps, then brought to the laboratory and kept at -72°C until the assay was scheduled. Those samples that did not appear visibly contaminated with blood or plaque were accepted.

Figure no 3: Collected sample transferred on aluminum foil

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eISSN: 2583-1194

Figure no 4: sample transferred to a centrifuge tube

Figure no 5: Freezer set at -72º

The assessment of biomarkers was carried out with the ELISA Kits: Enzyme-Linked Immunosorbent Assay.

SAMPLE PREPARATION AND ELISA

The collected GCF samples were mixed with 5 mL phosphate buffer (pH 7) and used for biochemical analysis. RANKL and OPG levels were assessed using commercially available ELISA kits. The prepared samples and standards were added to the respective wells of the microtitre plate and incubated. After washing, the respective biotinylated antibodies and streptavidin-HRP conjugate were added with subsequent incubation and washing steps. TMB substrate was then added, followed by stop solution, and the absorbance was measured at 450 nm using an ELISA reader.

SAMPLE SIZE ESTIMATION

The sample size was estimated using an effect size of 0.88, with 80% power and a 5% level of significance. Based on the calculation, a minimum sample size of 10 subjects was required.

STATISTICAL ANALYSIS

The data were analysed using SPSS version 26.0 and expressed as mean ± standard deviation (SD). Since samples from the maxilla and mandible were obtained from the same subjects, repeated-measures analysis of variance (ANOVA) was used to compare RANKL and OPG levels between the maxilla and mandible and across the three time points (baseline, 15 days and 1

month). Bonferroni post-hoc analysis was used for pairwise comparisons when a significant difference was observed. Gender was considered as a between-subject factor. A p value of <0.05 was considered statistically significant.

RESULTS

A total of 20 subjects were included in the study, comprising 10 males and 10 females. GCF samples were evaluated at baseline, 15 days, and 1 month following orthodontic force application.

RANKL levels

RANKL levels showed significant changes over time in both the maxilla and mandible ($p < 0.001$). In males, RANKL levels increased from baseline to 15 days in both regions, followed by a decrease at 1 month. The highest RANKL level in males was observed in the mandible at 15 days (0.047 ± 0.008). In females, RANKL levels showed significant changes over time in both regions, with an increase in the maxilla from baseline to 1 month and a progressive increase in the mesial mandibular region.

Table 1. RANKL levels in the maxilla and mandible at different time points

Region	Gender	Baseline (Mean ± SD)	15 days (Mean ± SD)	1 month (Mean ± SD)
Maxilla	Male	0.017 ± 0.005	0.036 ± 0.009	0.030 ± 0.002
Maxilla	Female	0.040 ± 0.010	0.045 ± 0.017	0.054 ± 0.021
Mandible	Male	0.020 ± 0.005	0.047 ± 0.008	0.035 ± 0.014
Mandible	Female	0.045 ± 0.009	0.045 ± 0.001	0.054 ± 0.018

OPG levels

OPG levels also showed significant time-dependent changes in both the maxilla and mandible ($p < 0.001$). In males, maxillary OPG increased from baseline to 15 days and subsequently decreased at 1 month. In the mandible, OPG showed a marked decrease at 15 days followed by an increase at 1 month. In females, OPG increased progressively in the maxilla, while mandibular OPG increased from baseline to 15 days and showed only a slight increase at 1 month.

Table 2. OPG levels in the maxilla and mandible at different time points

Region	Gender	Baseline (Mean ± SD)	15 days (Mean ± SD)	1 month (Mean ± SD)
Maxilla	Male	0.057 ± 0.010	0.072 ± 0.001	0.048 ± 0.018
Maxilla	Female	0.078 ± 0.026	0.087 ± 0.025	0.119 ± 0.038
Mandible	Male	0.087 ± 0.009	0.030 ± 0.007	0.058 ± 0.020
Mandible	Female	0.068 ± 0.034	0.095 ± 0.033	0.097 ± 0.023

RANKL/OPG ratio

The RANKL/OPG ratio demonstrated temporal and gender-related variations. At baseline, females showed higher ratios in both the maxilla and mandible compared with males. At 15 days, the highest ratio was observed in the male mandible (1.56), followed by a decrease at 1 month. This indicated a more pronounced early bone-resorptive response in the male mandible.

Table 3. RANKL/OPG ratio in the maxilla and mandible

Time	Gender	Maxilla	Mandible
Baseline	Male	0.30	0.23
	Female	0.51	0.66

15 days	Male	0.50	1.56
	Female	0.52	0.47
1 month	Male	0.62	0.60
	Female	0.45	0.56

OVERALL FINDINGS

Overall, significant time-dependent changes were observed in RANKL, OPG and the RANKL/OPG ratio during early orthodontic tooth movement. The most pronounced early increase in bone-resorptive activity was observed at 15 days, particularly in the mandibular region of males, as reflected by the RANKL/OPG ratio of 1.56. By 1 month, the ratio showed a tendency toward stabilization.

DISCUSSION

Orthodontic tooth movement involves remodeling of the periodontal ligament and alveolar bone, with the RANKL–OPG system playing an important role in regulating bone resorption.[9,16]

In the present study, RANKL levels showed significant changes over time in both the maxilla and mandible. In males, RANKL increased from baseline to 15 days, with the highest level observed in the mandible at 15 days, followed by a decrease at 1 month. Similar increases in RANKL following orthodontic force have been reported by Nishijima et al. and Barbieri et al.[12,16]

OPG levels also showed time-dependent changes. In males, mandibular OPG decreased at 15 days and increased again at 1 month, while females showed a more gradual increase, particularly in the maxilla. These changes may represent the regulatory response associated with the early bone-remodeling process.[7,13]

The RANKL/OPG ratio was highest in the male mandible at 15 days (1.56), suggesting a greater relative bone-resorptive response during the early phase. The ratio decreased at 1 month, indicating a tendency toward stabilization. Recent evidence also supports changes in the RANKL/OPG ratio following mechanical compression during orthodontic tooth movement.[1]

An important finding was the difference between the maxilla and mandible, with a more pronounced early response in the mandibular region in males. However, direct studies comparing biochemical bone metabolism between the two jaws are limited. GCF provides a minimally invasive method for evaluating such biochemical changes, and previous studies have demonstrated its usefulness for assessing biomarkers during orthodontic tooth movement.[3,5,11,12]

Overall, the findings suggest that bone metabolism changes dynamically during early orthodontic tooth movement, with a more pronounced early bone-resorptive response in the mandible, particularly in males.

LIMITATIONS

- Only Class I malocclusion cases were included; Class II and Class III malocclusions were not evaluated.
- The study did not assess the influence of tooth rotation on the biochemical response.
- The study evaluated biochemical changes in GCF but did not directly correlate them with the actual amount or rate of tooth movement.

FUTURE SCOPE

- Future studies can include larger sample sizes and patients with Class I, Class II and Class III malocclusions.
- The effect of tooth rotation and different types of tooth movement can be evaluated.
- More biomarkers involved in inflammation and bone remodelling can be studied along with RANKL and OPG.
- Longer follow-up with additional time points can help understand the changes throughout orthodontic treatment.

- Future studies can correlate GCF biomarker levels with the actual rate and amount of orthodontic tooth movement.

CONCLUSION

The present study showed significant changes in RANKL and OPG levels in GCF during early orthodontic tooth movement. The mandibular region, particularly in males, demonstrated a more pronounced early bone-resorptive response, as reflected by the higher RANKL/OPG ratio at 15 days. These findings suggest that bone metabolism may differ between the maxilla and mandible during orthodontic tooth movement. GCF analysis of RANKL and OPG may therefore be useful for assessing the biological response to orthodontic force.

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