

OPG and RANKL levels around miniscrew implants during orthodontic tooth movement

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Introduction: The aim of this study was to determine the peri-miniscrew implant crevicular fluid receptor activator of nuclear factor- κ B ligand (RANKL) and osteoprotegerin (OPG) levels around loaded and unloaded miniscrew implants at different time intervals. **Methods:** Twenty loaded and 16 unloaded miniscrew implants were included in this study. All miniscrew implants were placed bilaterally between the maxillary second premolars and first molars as anchorage units for canine distalization. Peri-miniscrew implant crevicular fluid was taken from the mesiobuccal aspects of the loaded and unloaded miniscrew implants before loading; at 24, 48, and 168 hours; and on day 30 after force application. Enzyme-linked immunosorbent assay kits were used to determine RANKL and OPG levels in the peri-miniscrew implant crevicular fluid samples. Wilcoxon, Mann-Whitney U, and Spearman correlation tests were used for statistical evaluations at the $P < 0.05$ level. **Results:** Although the total amount of OPG was not different between the groups, the total amount of RANKL was significantly elevated in the loaded miniscrew implant group ($P < 0.05$) at all time periods. Peri-miniscrew implant crevicular fluid volume was the highest at 48 hours in the loaded group. Also, the OPG/RANKL ratio in the peri-miniscrew implant crevicular fluid was significantly decreased in the loaded miniscrew implant group. **Conclusions:** The OPG and RANKL levels vary around loaded and unloaded miniscrew implants as a result of force application. (Am J Orthod Dentofacial Orthop 2013;144:203-9)

Skeletal anchorage with dental implants,¹ miniplates,² miniscrews,³ and microscrews⁴ provides absolute anchorage for tooth movement. Miniscrew implants have many benefits, such as immediate or early loading, ease of placement and removal, and relatively low cost.⁴ These advantages have expanded the use of miniscrew implants for various orthodontic treatments.⁵ Both clinical and experimental studies have demonstrated that miniscrew implants can provide

sufficient and stable anchorage for tooth movement during the entire orthodontic therapy.⁶

Mechanical stimulation can initiate and promote bone remodeling.⁷ Osteoblasts and osteoclasts are specialized cells responsible for bone formation and resorption, respectively. Many investigators have studied the interaction between these 2 cell types.⁸ Recently, osteoprotegerin (OPG) and receptor activator of nuclear factor- κ B ligand (RANKL) have been shown to play important roles in bone remodeling.^{8,9} OPG is a member of the tumor necrosis factor receptor family and a soluble decoy receptor against RANKL. It is produced by osteoblasts and other cells and has been found to be a key factor in the inhibition of osteoclast differentiation and activation.¹⁰ In contrast, RANKL, which is expressed on the surface of osteoblasts, activates differentiation of preosteoclastic cells into mature osteoclasts and thus promotes bone resorption.¹¹ The activation of clastic cells is regulated by the activation of the receptor activator of NF- κ B. RANKL binds to receptor activator of NF- κ B and stimulates differentiation and signaling pathways in clastic cell precursors. Binding of OPG to RANKL inhibits the genesis of clastic cells, thus preventing RANKL linkage to receptor activator of NF- κ B.¹² Recent studies have shown that mechanical strain plays an important role in the regulation of OPG synthesis and RANKL expression.^{13,14}

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Peri-miniscrew implant crevicular fluid (MICF) is the inflammatory transudate that flows out via the miniscrew implant crevice.¹⁵ The biomarkers in the MICF are used to assess the host's response to mechanical forces. Similar to a natural tooth, as the force is applied, inflammatory reactions begin in tissues around miniscrew implants.^{16,17} In contrast to natural teeth, inflammatory reactions should be strictly limited by preventing weakness in the stability.

In tooth movement as mechanical forces are applied, periodontal ligament cells produce cytokines during bone remodeling. Likewise, similar mechanisms take place in MICF when a force is exerted. The composition of MICF is similar to that of gingival crevicular fluid, and both are affected by bone modeling procedures. Whereas changes in gingival crevicular fluid lead to the destruction of periodontal tissues, changes in MICF cause destruction in peri-implant structures. Hence, it can be speculated that the elevations of cytokines and enzymes in MICF reflect biologic responses induced by mechanical stress.¹⁸

To our knowledge, no published study has evaluated the RANKL and OPG levels around miniscrew implants as a response to orthodontic force application. Therefore, the purposes of this study were to determine MICF volume, RANKL and OPG levels, and the OPG/RANKL ratio, and to test the null hypothesis that there are no statistically significant differences in MICF RANKL, OPG levels, and OPG/RANKL ratio between loaded and unloaded miniscrew implants at different time intervals.

MATERIAL AND METHODS

A power analysis established by G*Power software (version 3.0.10; Franz Faul, Universität Kiel, Kiel, Germany), based on a 1:1 ratio among the groups, with a sample size of 37 miniscrew implants, would give more than 85% power to detect significant differences with a 0.45 effect size and at the $\alpha = 0.05$ significance level.

Twenty patients who required bilateral maxillary first premolar extractions and canine distalizations as a part of their orthodontic treatment were included in this study. All patients were in good general health with healthy periodontiums and generalized probing depths not exceeding 3 mm, with no radiographic evidence of periodontal bone loss. Patients who had antibiotic therapy within the past 6 months and used anti-inflammatory drugs in the month before the study did not participate. Written informed consents were obtained from all patients or the parents of those under 18 years of age.

The patients were randomly divided into 2 groups, loaded and unloaded miniscrew implants, according to the loading pattern of their implants. The simple randomization method was used, with the software producing

Table I. Sex and age distributions of the groups

	Loaded	Unloaded
Age (y)	18.15 ± 3.06	17.75 ± 0.68
Sex	3 female, 7 male	3 female, 5 male

the randomization lists (version 15.0; SPSS, Chicago, Ill). Two patients in the unloaded miniscrew group were excluded because of improper oral hygiene. The mean ages of the loaded (3 male, 7 female) and unloaded (3 male, 5 female) miniscrew implant groups were 18.15 ± 3.06 and 17.75 ± 0.68 years, respectively (Table I).

Initially, fixed preadjusted edgewise brackets with 0.018 × 0.025-in slots were placed in the maxillary arch. After leveling, a 0.016 × 0.022-in stainless steel archwire was placed, and the second maxillary premolars and the first molars were ligated together before canine distalization. A total of 36 titanium miniscrew implants (8 mm long, 1.6 mm diameter; Mitos, Istanbul, Turkey) were placed bilaterally into the interradicular bone between the maxillary second premolars and first molars in the attached gingiva below the mucogingival junction. To reduce root contact, the implants were placed in an oblique direction buccolingually, 30° to 40° to the long axis of the teeth in the maxillary posterior area, as described by Park.¹⁹ In the loaded miniscrew group, a 150-g distalization force delivered by 7-mm Sentalloy closed-coil springs (GAC International, Bohemia, NY) was applied horizontally between the miniscrew and the canine immediately after the insertion of the miniscrew implants. In the unloaded miniscrew group, the loading was performed 1 month later. MICF volume, total amounts and concentrations of OPG and RANKL, and the OPG/RANKL ratio were determined.

MICF sampling from both groups at each time point was performed by a periodontist (O.C.) according to the method of Uematsu et al²⁰ with a slight modification. The samples were obtained before loading; at 24, 48, and 168 hours later; and on day 30 after force application. The timeline of the study is shown in Figure 1.

After removal of plaque around the miniscrew implants, MICF samples were collected using paper strips (OraFlow, Plainview, NY) from the mesiobuccal aspects of the miniscrew implants. Each site was gently air-dried and isolated with cotton rolls, and paper strips were inserted into the crevice until mild resistance was felt. The paper strips were left for 30 seconds and transferred to an electronic gingival fluid measuring device (Periotron 8000; OraFlow) for volume determination. These samples were stored at -20°C and then transferred to -80°C until analysis. After 30 seconds of vortexing and 20 minutes of shaking, the strips were removed, and 200-μL eluted samples were assayed with

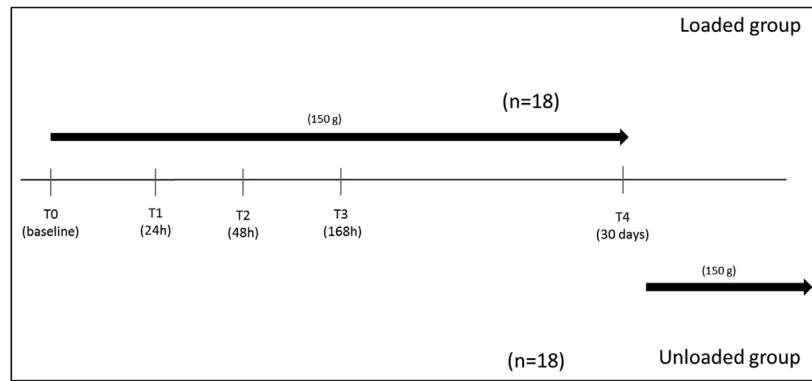


Fig 1. Timeline of the study.

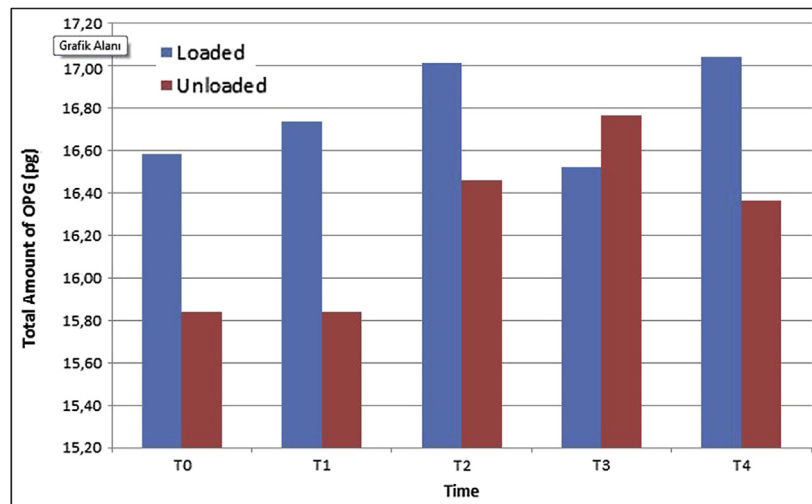


Fig 2. Changes in the total amounts of OPG for both groups at all time periods.

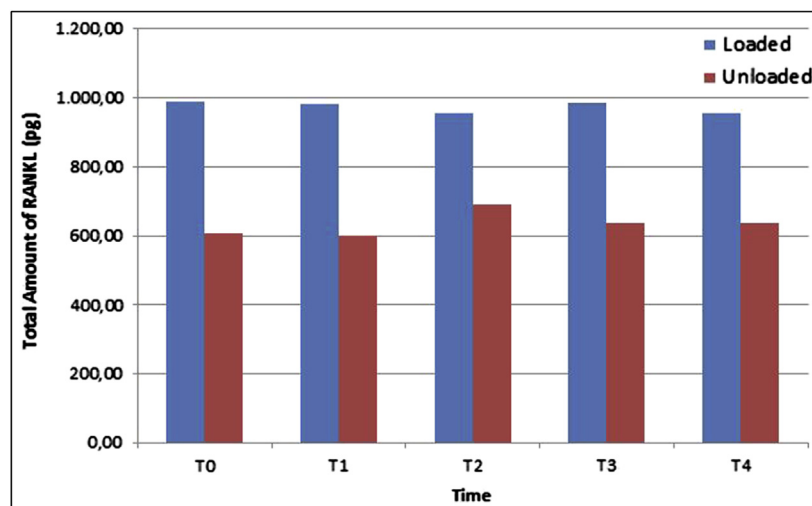


Fig 3. Changes in the total amounts of RANKL for both groups at all time periods.

Table II. Descriptive statistics and intragroup comparisons of total amounts and concentrations of OPG and RANKL at all time points

	T0				T1			
	Min	Median	Max	P	Min	Median	Max	P
Total amount of OPG (pg)								
Loaded	11.34	16.58	41.43	NS	13.44	16.74	24.78	NS
Unloaded	12.58	15.84	19.79		13.69	15.84	18.56	
Total amount of RANKL (pg)								
Loaded	455	988.75	1293	*	437.5	982.5	1317	*
Unloaded	417.5	607.5	1310		460	601.25	1157	
Concentration of OPG (pg/ μ L)								
Loaded	37.81	140.7	217.6	NS	30.98	72.74	191.7	NS
Unloaded	27.64	130.69	171.98		52.19	81.06	168.9	
Concentration of RANKL (pg/ μ L)								
Loaded	2868.75	5168.75	12925	NS	1168	4631.25	12800	NS
Unloaded	933	4762.5	13100		1683	3935.42	7750	

T0, Before loading; T1, 24 hours later; T2, 48 hours later; T3, 168 hours later; T4, 30 days later; Min, minimum; Max, maximum; NS, not significant.

* $P < 0.05$.

enzyme-linked immunosorbent assay (ELISA) kits. MIFC collection was standardized so that the loaded and unloaded sites and the subjects could be compared. Samples contaminated with saliva or blood were excluded. MIFC samples were taken before all other clinical examinations were performed to prevent an increase in fluid volume.

The amounts of OPG and RANKL in the MIFC samples were determined using commercially available human-specific ELISA in accordance with the manufacturers' instructions (total RANKL ELISA kit; Raybiotech Inc, Norcross, Ga; OPG ELISA kit, USCN Life Science [USCNK] Inc, Houston, Tex). These assays measured the total levels of RANKL and OPG in the MIFC, including both their unbound, free forms, and their OPG and RANKL complex forms. Calculation of the RANKL and OPG concentrations in each MIFC sample was performed by dividing the total amount of RANKL or OPG by the volume of the sample: RANKL or OPG concentration (pg/ μ L) = total RANKL or OPG (pg)/volume (μ L).

Statistical analysis

All statistical analyses were performed using software (SPSS). The Shapiro-Wilks normality test and the Levene variance homogeneity test were applied to the data, which did not show a normal distribution; there was no homogeneity of variances between groups. Intragroup comparisons were evaluated using the nonparametric Wilcoxon test. The statistical significance of intergroup differences was further assessed with the Mann-Whitney U test. The correlation among the MIFC parameters was evaluated with the Spearman rank correlation test. The statistical significance level was set at $P < 0.05$.

RESULTS

All miniscrew implants survived until the end of study. There was a statistically significant increase in the total amount of OPG only at 168 hours ($P < 0.05$; Fig 2) in the unloaded miniscrew implant group, with no statistically significant differences in the loaded miniscrew implant group between all time periods compared with the baseline measurements. Similarly, there was no statistically significant difference in the total amount of RANKL in the groups at all time periods (Fig 3) in comparison with the baseline. The results of the analysis indicated no statistically significant differences between the loaded and unloaded miniscrew implant groups for total amounts of OPG ($P > 0.05$), but the total amounts of RANKL were significantly elevated in the loaded miniscrew implant group ($P < 0.05$) with respect to the unloaded miniscrew implant group at all time periods. Both RANKL and OPG concentrations decreased at 48 hours in the loaded miniscrew implant group (Table II). The volume of MIFC was elevated only at 48 hours in the loaded group (Fig 4). Although the MIFC OPG/RANKL ratio remained unchanged in the loaded miniscrew implant group, it increased in the unloaded group at the end of the observation period (Fig 5).

Based on these results, the null hypothesis was rejected.

DISCUSSION

The effectiveness and clinical application of the miniscrew implants have already been reported in literature, but less is known about the biomolecular level that affects implant stability.^{19,21,22} To keep the miniscrew

Table II. Continued

T2				T3				T4			
Min	Median	Max	P	Min	Median	Max	P	Min	Median	Max	P
12.76	17.01	31.69	NS	10.85	16.52	22.99	NS	13.13	17.04	22.62	NS
13.75	16.46	18.74		13.56	16.77	21.58		14.80	16.36	26.63	
382.5	953.75	1310	*	490	986.25	1245	*	587.5	956.25	1327	*
447.5	691.25	1122		377.5	637.5	1227.5		432.5	637.50	987.5	
16.41	43.97	112.19	*	13.56	73.66	172.6	NS	19.26	77.52	204.65	NS
28.97	74.13	173.23		28.15	75.67	167.68		53.63	87.38	188.03	
1162	2471.25	6550	NS	1140	4212.5	10625	NS	1447	4456.25	13100	NS
841	2568.75	7475		693	3187.5	9225		2162	2912.5	9875	

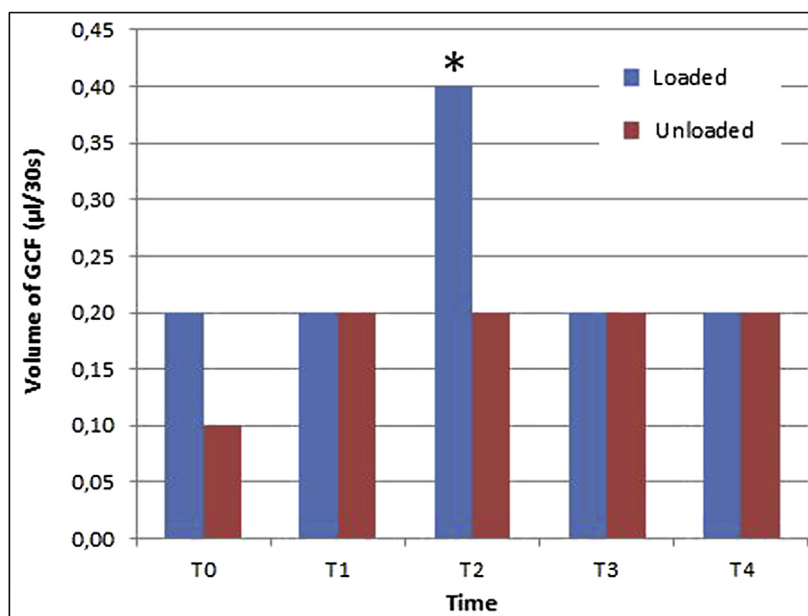


Fig 4. Changes in MICF volumes for both groups at all time periods. **P* <0.05.

implants stable for proper orthodontic anchorage, tissue reactions to the applied forces should be known. Although many studies have evaluated the proinflammatory cytokines in MICF, monitoring the health status of miniscrew implants,^{16,17} our study is the first to determine the OPG and RANKL axis, which is a key regulator of osteoclastogenesis and bone remodeling around the miniscrew implants used for direct anchorage.²³

In contrast to dental implants, orthodontic miniscrews can be loaded immediately,^{22,24} and immediate

loading with orthodontic forces can be performed without complications.^{25,26} Only a few studies, mostly on animals, have dealt with the tissue reactions to immediate loading of miniscrew implants.^{6,27,28} In our study, miniscrew implants were placed in the attached gingiva to prevent tissue inflammation. Insertion of the miniscrews below the mucogingival junction allowed a relatively self-cleaning area by physiologic movements. As a result, all miniscrew implants survived until the end of the observation period without complications.

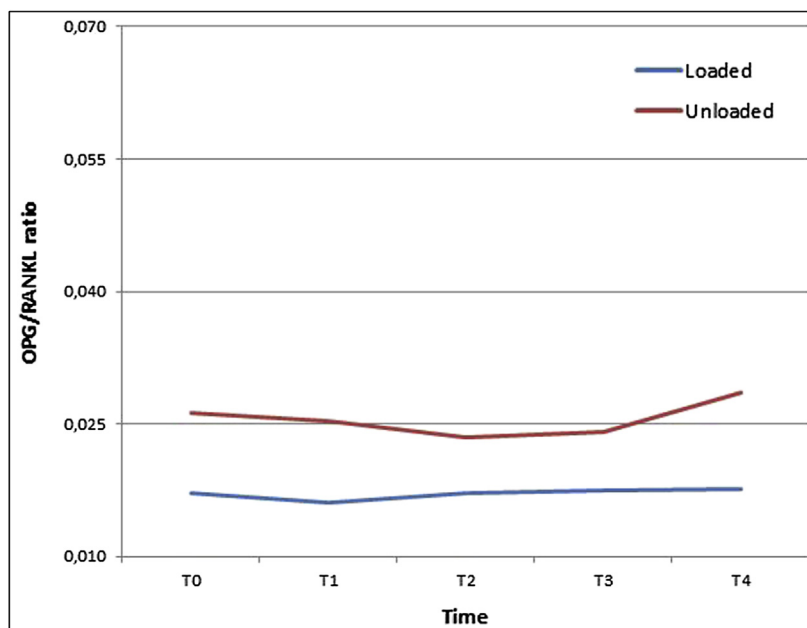


Fig 5. Changes in MICF OPG/RANKL ratios for both groups at all time periods.

MICF volume has been shown to be correlated with inflammatory state.^{16,17} Thus, we compared the mean volumes of MICF taken from both paper strips at each time point. The OPG and RANKL levels in the MICF were determined similarly to the methods used in previous studies.^{16,29,30} Our results showed that MICF volume was elevated only at 48 hours in the loaded group. Because of this increase in MICF, it is logical to observe that both RANKL and OPG concentrations showed relative decreases at 48 hours in the loaded miniscrew implant group.

Although the total amount of OPG remained unchanged, horizontally applied force altered the amount of RANKL in the loaded miniscrew implant group at all periods. Increases in RANKL can reflect inflammation and might be assumed as potential markers of bone destruction in peri-implant tissues. Changes in the total amount of RANKL can be attributed to displacement toward the direction of force under orthodontic loading. Wang and Liou³¹ reported that miniscrew implants were subjected to displacement under orthodontic loading, and the amount of displacement was correlated to the length of the loading period, although the implants remained stable without detectable mobility or loosening. Also, in a 3-dimensional study, it was concluded that movement of miniscrew implants is expected during orthodontic loading.³²

Normal bone turnover and stable bone mass depend on the balance between OPG and RANKL.³³ It was recently shown in vivo that the ratio of the concentrations

of RANKL to OPG in the gingival crevicular fluid was significantly higher in patients with periodontal disease than in healthy subjects.³⁴ Grimaud et al³⁵ demonstrated that imbalances in the OPG and RANKL systems might play roles in bone resorption mechanisms. The ratio of OPG and RANKL might be the ultimate determinant of bone resorption.³⁶ Interestingly, our results showed that the OPG/RANKL ratio had a slight increase with respect to the level before loading in the unloaded miniscrew implant group. On the other hand, this ratio remained almost unchanged in the loaded miniscrew implant group at the end of the observation period. The increase in the unloaded group might be attributed to the acute inflammation resulting from the placement of the miniscrew implants and the remodeling process. Vande Vannet et al³⁷ indicated that miniscrew implants used for temporary anchorage in orthodontics partially osseointegrate. Also in the loaded group, the stable ratio could reflect that the miniscrew implants we used were clinically healthy.

However, these results need to be confirmed with longer study periods; also, further studies focused on different amounts of forces should be performed to clarify the interaction between force and peri-implant tissues.

CONCLUSIONS

The OPG and RANKL levels vary as a result of force application. Also, the ratio of OPG and RANKL is stable around loaded miniscrew implants.

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