Original Article

A comparative evaluation of osteoprotegerin and receptor activator of nuclear kappa B ligand in gingival crevicular fluid during canine retraction with elastomeric chain and nickel titanium coil spring: An *in vivo* study

ABSTRACT

Objective: The objective of this study is to evaluate and compare the level of receptor activator of nuclear kappa B ligand (RANKL) and osteoprotegerin (OPG) in gingival crevicular fluid (GCF) during canine retraction with nickel-titanium (NiTi) coil spring and elastomeric chain.

Materials and Methods: Ten patients between the ages of 13–17 years requiring canine distalization after first premolar extraction were selected. NiTi coil spring was placed on one side and elastomeric chain on the contralateral side. GCF sampling was done at baseline, 1 h, 24 h, 168 h, 1 month from the distal site of the test teeth after the application of compressive force. RANKL and OPG concentration were detected by enzyme-linked immunosorbent assay.

Results: In the NiTi coil spring side, the RANKL levels in GCF increased and OPG levels decreased in a time-dependent manner. Increase in RANKL and decrease in OPG were significant when compared with the baseline. In the elastomeric chain side, the RANKL levels were significantly higher and the OPG levels significantly lower at 24 h, the RANKL levels showed a significant decrease and OPG levels showed a significant increase from 24 h to 1 month time intervals. The RANKL and OPG levels in GCF when compared individually to the NiTi coil spring and elastomeric chain showed a significant difference at 24 h, 168 h, and 1 month after initiation of compressive force.

Conclusion: The result suggests that RANKL and OPG expression during orthodontic tooth movement varies at different time intervals and according to the type of force delivery system.

Keywords: Elastomeric chain, gingival crevicular fluid, nickel titanium coil spring, orthodontic tooth movement, osteoprotegerin, receptor activator of nuclear kappa B ligand

INTRODUCTION

Orthodontic tooth movement is a continuous and balanced process characterized by bone deposition and bone resorption respectively on tension and compression sites.[1] Complete orthodontic tooth movement because of alveolar bone remodeling involves several processes over a certain period. There are three phases in an orthodontic tooth movement, an initial displacement phase that reflects the viscoelastic properties of the tooth-supporting structures, a delay phase or lag phase characterized by no movement and postlag phase with linear tooth movement. Most of the tooth movement whether gradual or sudden increase occurs during the acceleration or linear phase when alveolar remodeling occurs.[2]

Gingival crevicular fluid (GCF) is an exudate that precisely reflects the biological events in the periodontium in response

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Ejaz Salim Khan, Abhishek Ranjan, N Raghunath
Department of Orthodontics, JSS Dental College and Hospital, JSS University, Mysore, Karnataka, India

Address for correspondence: Dr. Ejaz Salim Khan, Department of Orthodontics, JSS Dental College and Hospital, JSS University, Mysore, Karnataka, India.
E-mail: ejazsalimkhan1977@gmail.com

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to mechanical stimuli from the orthodontic force and may be used to detect the level of certain biomarkers.[3,4] Biologically, active substances called the biomarkers are expressed by cells within the periodontium in response to mechanical stimuli from orthodontic appliances. Biologically active substances are classified as biomarkers of inflammation, bone resorption, cell death, and bone deposition and mineralization were studied in GCF during orthodontic tooth movement.[1] Because of their important roles in tooth movement and even in tissue damage, monitoring the levels of certain biochemical mediators during orthodontic treatment may be useful.

Receptor activator of nuclear kappa B ligand (RANKL) is a membrane-associated Tumor necrosis factor (TNF) Ligand family, which was found to be responsible for induction of osteoclastogenesis. It is expressed in the plasma membrane of osteoblast and stromal cells.[5]

Osteoprotegerin (OPG) is also known as osteoclastogenesis inhibitory factor; it is a cytokine receptor and a member of the TNF family. OPG is a decoy receptor produced from osteoblastic lineage in competition with receptor activator of nuclear kappa B (RANK) for RANKL.[6,7]

Preadjusted fixed orthodontic appliances commonly utilize sliding mechanics for space closure with force delivery systems such as elastomeric chain, nickel titanium (NiTi) coil springs, or intraoral elastics.[8] The synthetic elastomeric chain was introduced in the 1960s and has been in widespread use since then. Elastomeric chains provide light continuous forces mainly during canine retraction after premolar extraction. The Stainless steel coil springs were introduced in 1930s and NiTi coil springs are used more recently. Increasingly, NiTi coil springs are used for space closure as they are thought to retain more force over a given time period and to provide a constant force.

There is a need to evaluate the levels of biomarkers at a different phase of tooth movement with different force delivering systems to understand the biomechanics of tooth movement. The present study is aimed to measure the level of RANKL and OPG in GCF of young patients undergoing orthodontic canine retraction with NiTi coil spring and elastomeric chain. The study is also aimed to compare the level of RANKL and OPG between NiTi coil spring and elastomeric chain.

MATERIALS AND METHODS

In the present study, 10 patients of age group 13–17 years, irrespective of gender who needed canine retraction and first premolar extraction as a part of fixed orthodontic treatment were selected from the outpatients in the Department of Orthodontics and Dentofacial Orthopedics, JSS Dental College and Hospital, JSS University, Mysore.

Materials used for preparation of samples

- 100 μL phosphate buffered saline
- Volumetric microcapillary pipette of 5 μL capacities.

Equipment used for measurement

- Commercially available ELISA kit.

Method of collection of data

- Nonprobability purposive sampling
- Ten patients satisfying the inclusion criteria were selected for the study.

Inclusion criteria

1. Patients requiring upper canine retraction after first premolar extraction as part of orthodontic treatment
2. General good health status
3. Good oral hygiene
4. Clinically healthy periodontal tissues with probing depth $\leq$3 mm, with no radiographic evidence of periodontal bone loss
5. No antibiotic therapy in the past 90 days
6. Nonusage of anti-inflammatory drugs in previous 30 days.

Exclusion criteria

1. Periodontally compromised patients
2. Patients with oral manifestations of a disease or a chronic debilitating disease
3. Smokers
4. Pregnant and nursing mothers.

Methodology

1. In the selected patients, 0.022” slot preadjusted edgewise appliance McLaughlin, Bennett, and Trevisi (MBT) prescriptions was used after upper right and left 1st premolar extractions as a part of orthodontic treatment. The selected patients underwent orthodontic treatment with sequential wire changes until 0.019” × 0.025” stainless steel wire were placed [Figure 1]. One month after the placement of 0.019 × 0.025” stainless steel wire, canine retraction was started with NiTi coil spring (200 g forces) on the upper right side and elastomeric chain (200 g forces) on the upper left side
2. GCF sampling was done 1 h before the commencement of treatment and at 1 h, 24 h, 168 h, and 1 month after the application of compressive orthodontic force at the test site [Figures 1-10]
3. The experimental tooth was isolated with cotton rolls

and supragingival plaque if any was removed with a curette, without touching the marginal gingiva
4. The sites were gently dried with an air syringe, and a saliva ejector was used to avoid any salivary contamination
5. GCF samples were collected from the distocervical gingival margin of the upper canines before any other clinical procedures to avoid blood contamination [Figure 7]
6. GCF samples were obtained by placing calibrated, the volumetric microcapillary pipette of an internal diameter of 1.1 mm with a capacity of 15 μL extra crevicular over test sites. From each test site, a standardized volume of 10 μL of GCF was collected [Figure 4]
7. The microcapillary pipette contaminated with saliva or blood was discarded
8. The absorbed GCF was eluted from the microcapillary pipette in 100 μL phosphate-buffered saline (pH 7.2–7.6)
9. The eluted samples were stored in polypropylene tubes at −20°C before analysis [Figure 9]
10. The samples were analyzed with the enzyme-linked immune sorbent assay ELISA technique [Figures 5,6 and 8-10].

Statistical analysis

Descriptive statistics, *t*-test-independent samples, *t*-test-paired samples, and repeated measure ANOVA. The data were collected and analyzed using the SPSS version 16.0 for Windows OS.

RESULTS

A significant difference was found in RANKL levels during orthodontic canine retraction by NiTi Coil Spring from baseline to 1 month, from 1 h to 1 month, from 24 h to 1 month, and between 168 h and 1 month ($P \leq 0.05$) [Table 1]. A significant difference was found in mean RANKL level from baseline to 1 h, 24 h, 168 h, and 1 month ($P \leq 0.05$) [Table 1]. A significant difference was found in mean RANKL levels from 1 h to 24 h, 168 h, and 1 month ($P \leq 0.05$) [Table 1]. A significant difference was found in mean RANKL levels from 24 h to 168 h and 1 month ($P \leq 0.05$) [Table 1]. A significant difference was found in mean RANKL levels from 168 h to 1 month ($P \leq 0.05$) [Table 1].

A statistical significant difference was found in RANKL level during orthodontic canine retraction by elastomeric chain from baseline to 1 month, from 1 h to 1 month, and from 24 h to 1 month and between 168 hours and 1 month ($P \leq 0.05$) [Table 2]. A significant difference was found in mean RANKL levels from baseline to 1 h, 24 h, 168 h, and 1 month ($P \leq 0.05$) [Table 2]. A significant difference was found in mean RANKL levels from 1 h to 24 h, 168 h, and 1 month ($P \leq 0.05$) [Table 2]. A significant difference was found in mean RANKL levels from 24 to 168 h and 1 month ($P \leq 0.05$) [Table 2]. A significant

Table 1: Paired *t*-test for receptor activator of nuclear kappa B ligand level in gingival crevicular fluid during orthodontic canine retraction by nickel titanium coil spring at different time intervals

Canine retraction	GCF sampling	Paired differences Mean	*t*	df	Significant (two-tailed)
Pair 1	Baseline to 1 h	−114.92	−10.56	9	0.000
Pair 2	Baseline to 1 day	−270.15	−28.95	9	0.000
Pair 3	Baseline to 1 month	−309.07	−17.58	9	0.000
Pair 4	Baseline to 1 month	−416.95	−19.81	9	0.000
Pair 5	1 h to 1 day	−155.22	−17.58	9	0.000
Pair 6	1 h to 1 week	−194.14	−12.05	9	0.000
Pair 7	1 h to 1 month	−302.03	−15.47	9	0.000
Pair 8	1 day to 1 week	−38.917	−2.342	9	0.044
Pair 9	1 day to 1 month	−146.80	−8.713	9	0.000
Pair 10	1 week to 1 month	−107.88	−6.699	9	0.000

Table 2: Paired *t*-test for receptor activator of nuclear kappa B ligand level in gingival crevicular fluid during orthodontic canine retraction by elastomeric chain at different time intervals

Canine retraction	GCF sampling	Paired differences Mean	*t*	df	*P*
Pair 1	Baseline to 1 h	−96.046	−10.71	9	0.000
Pair 2	Baseline to 1 day	−203.85	−13.29	9	0.000
Pair 3	Baseline to 1 week	−144.77	−8.937	9	0.000
Pair 4	Baseline to 1 month	−53.458	−3.913	9	0.004
Pair 5	1 h to 1 day	−107.80	−6.069	9	0.000
Pair 6	1 h to 1 week	−48.724	−3.738	9	0.005
Pair 7	1 h to 1 month	42.587	3.114	9	0.012
Pair 8	1 day to 1 week	59.084	4.076	9	0.003
Pair 9	1 day to 1 month	150.39	8.095	9	0.000
Pair 10	1 week to 1 month	91.311	8.756	9	0.000

Table 3: Paired *t*-test for osteoprotegerin level in gingival crevicular fluid during orthodontic canine retraction by nickel titanium coil spring at different time intervals

Canine retraction	GCF sampling	Paired differences Mean	*t*	df	Significant (two-tailed)
Pair 1	Baseline to 1 h	155.68	10.873	9	0.000
Pair 2	Baseline to 1 day	308.16	14.805	9	0.000
Pair 3	Baseline to 1 week	333.29	15.209	9	0.000
Pair 4	Baseline to 1 month	374.30	14.211	9	0.000
Pair 5	1 h to 1 day	152.47	6.006	9	0.000
Pair 6	1 h to 1 week	177.60	7.739	9	0.000
Pair 7	1 h to 1 month	218.61	8.362	9	0.005
Pair 8	1 day to 1 week	25.120	1.800	9	0.105
Pair 9	1 day to 2 weeks	66.133	4.289	9	0.002
Pair 10	1 week to 1 month	41.008	4.349	9	0.002

difference was found in mean RANKL levels from 168 h to 1 month ($P \leq 0.05$) [Table 2].

A significant difference was found in OPG levels during orthodontic canine retraction by NiTi Coil Spring from baseline to 1 month, 1 h to 1 month, 24 h to 1 month, and 168 h to 1 month ($P \leq 0.05$) [Table 3]. A significant difference was found in mean OPG level from baseline to 1 h, 24 h, 168 h, and 1 month ($P \leq 0.05$) [Table 3]. A significant difference was found in mean OPG level from 1 h to 24, 168 h, and 1 month ($P \leq 0.05$) [Table 3]. No significant difference was found in mean OPG level from 24 h to 168 h ($P \geq 0.05$) [Table 3]. A significant difference was found in mean OPG level from 24 h to 1 month ($P \leq 0.05$) [Table 3]. A significant difference was found in mean OPG level from 168 h to 1 month ($P \leq 0.05$) [Table 3].

A significant difference was found in OPG level during orthodontic canine retraction by elastomeric chain from baseline to 1 month, 1 h to 1 month, 1 day to 1 month, and 1 week to 1 month ($P \leq 0.05$) [Table 4]. A significant difference was found in mean OPG levels from baseline to 1 h, 24 h, 168 h week, and 1 month ($P \leq 0.05$) Table 4. A significant difference was found in mean OPG levels from 1 h to 24 h, 168 h, and 1 month ($P \leq 0.05$) [Table 4]. A significant difference was found in mean OPG levels from 24 h to 168 h and 1 month ($P \leq 0.05$) [Table 4]. A significant difference was found in mean OPG levels from 168 h to 1 month ($P \leq 0.05$) [Table 4].

No significant difference was found in RANKL levels in GCF between NiTi coil spring and elastomeric chain at 1 h ($P \geq 0.05$) [Table 5].

A significant difference in was found in RANKL levels in GCF between NiTi coil spring and elastomeric chains at 24 h, 168 h, and 1 month ($P \leq 0.05$) [Table 5].

No significant difference was found in OPG level in GCF between NiTi coil spring and elastomeric chain at 1 h ($P \geq 0.05$) [Table 6].

Significant difference was found in OPG level in GCF between NiTi coil spring and elastomeric chain at 24 h, 168 h, and 1 month ($P \leq 0.05$) [Table 6].

Table 4: Paired *t*-test for osteoprotegerin level in gingival crevicular fluid during orthodontic canine retraction by elastomeric chain at different time intervals

Canine retraction	GCF sampling	Paired differences Mean	*t*	df	*P*
Pair 1	Baseline to 1 h	164.84	11.42	9	0.000
Pair 2	Baseline to 1 day	258.35	12.23	9	0.000
Pair 3	Baseline to 1 week	109.94	6.289	9	0.000
Pair 4	Baseline to 1 month	63.14	4.430	9	0.002
Pair 5	1 h to 1 day	93.50	3.648	9	0.005
Pair 6	1 h to 1 week	−54.90	−3.28	9	0.009
Pair 7	1 h to 1 month	−101.70	−5.820	9	0.000
Pair 8	1 day to 1 week	−148.41	−9.194	9	0.000
Pair 9	1 day to 1 month	−195.21	−14.65	9	0.000
Pair 10	1 week to 1 month	−46.80	−5.300	9	0.000

Table 5: *T*-test for receptor activator of nuclear kappa B ligand level in gingival crevicular fluid during orthodontic canine retraction with nickel titanium coil spring and elastomeric chain at different time intervals

	t	df	*P*
Baseline	−0.038	18	0.970
1 h	−0.796	18	0.436
24 h	−2.692	18	0.015
168 h	−5.425	18	0.000
1 month	−11.793	18	0.000

Table 6: *T*-test for osteoprotegerin level in gingival crevicular fluid during orthodontic canine by nickel titanium coil spring and elastomeric chain at different intervals

	t	df	*P*
Baseline	−0.060	18	0.953
1 h	−0.201	18	0.843
1 day	2.214	18	0.048
1 week	8.462	18	0.000
2 weeks	12.061	18	0.000

DISCUSSION

Orthodontic tooth movement is a process characterized by bone remodeling with bone deposition on tension side and bone resorption on the compression side.[1] Orthodontic tooth movement can be controlled by the magnitude, direction and duration of the applied force and the biologic response from the periodontal cells.[2] The force applied to the teeth will cause a change in the microenvironment around the PDL due to alterations in the blood flow leading to the secretions of different inflammatory mediators such as cytokines, growth factors, neurotransmitters, colony stimulating factors and arachidonic acid metabolites.[3] The secretions of these inflammatory factors result in bone remodeling.

RANKL is a member of TNF ligand superfamily 11 (TNFSF11), it is expressed in the plasma membrane of osteoblast and stromal cells. RANKL binds directly to its receptor RANK and induces osteoclast differentiation and stimulates bone resorptive activity.[4,5]

OPG is a cytokine receptor and a member of a tumor receptor superfamily (TNFSF11B). OPG is produced by human

Figure 1: Arch wire

Figure 3: Dontrix gauge

Figure 5: Elisa kit

periodontal ligament cells and gingival fibroblasts. OPG is a naturally occurring inhibitor of osteoclastic differentiation, in turn promoting osteogenesis.[7] OPG acts as an inhibitory factor of Osteoclastogenesis by competing with RANK, which is the receptor for RANKL.[6] OPG functions as a decoy receptor for RANKL by binding to it with high affinity and blocks RANKL from interacting with RANK thereby preventing the formation RANK–RANKL complex necessary to induce osteoclastogenesis. RANKL and OPG signaling, as well as regulation of their expression, may play an important role in bone remodeling during orthodontic tooth movement.[7] Biomechanical analysis of the GCF has provided a no invasive model for investigating the cellular response of the underlying periodontal ligament during orthodontic tooth movement *in vivo*. The most commonly used force delivery system in sliding mechanics is elastic auxiliaries.[8,9] Elastic auxiliaries are relatively consistent

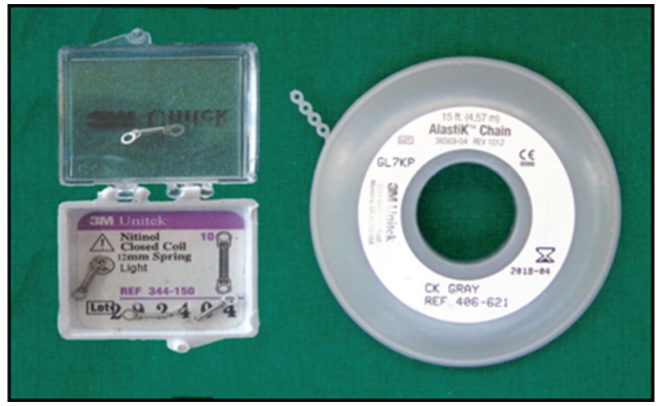

Figure 2: Nickel titanium coil spring and elastomeric chain

Figure 4: Micro capillaries

Figure 6: Application of force

in producing tooth movement with the drawback of force decay, so the initial forces must often be greater than is desirable.[10] NiTi alloys have gained substantial popularity since their introduction, especially with their two unique properties of super elasticity[11,12] and the shape memory phenomenon.[13]

The experiment design of the present study is to evaluate the expression of RANKL and OPG using NiTi coil spring on one side and elastomeric chain on the opposite side and to compare the level of OPG and RANKL between the two different force applications.

The present study found that on the Ni-Ti coil spring side, a significant increase in RANKL level and a significant decrease

Figure 7: Collection of sample

Figure 9: Incubator

in OPG levels on Ni-Ti coil spring side at 1 hour after the initiation of compressive force. The increased levels of RANKL and decreased levels of OPG were maintained at 24 hours, 168 hours and 1 month after the orthodontic force initiation. Nishijima *et al*[14] in an *In vitro* study has indicated that the compression force significantly increased the secretion of RANKL and decreased that of OPG in PDL in a time-dependent manner. Yijin *et al*[15] have demonstrated that protein inflammatory cytokine TNF-α were up-regulated during early stages of orthodontic tooth movement under continuous orthodontic force. The present study is in accordance with the above studies.

On the elastomeric chain side, there is a significant increase in mean RANKL level and a significant decrease in OPG levels in GCF at 24 h after the initiation of compressive orthodontic force. The level of RANKL was significantly decreased and OPG level significantly increased after 24 h and decreased levels of RANKL and increased level of OPG was maintained at 168 h and 1 month after initiation of compressive force. Kawasaki *et al*.[16] have done a study to compare the level of OPG in juvenile and adult patient during orthodontic tooth movement. They found significantly lower OPG and significant higher RANKL levels after 24 h in both groups, a greater decrease of OPG and a greater increase in RANKL level was found in the juvenile group. Nishijima *et al*.[14] in an *in vivo* study has investigated RANKL and OPG expression during 1-week period. They found a significant decrease in

Figure 8: Storage freezer (−20°)

Figure 10: Micro plate reader

OPG level and significant increase of RANKL level at 24 h after orthodontic force initiation. The present study result is in accordance with the above findings.

The present study found that when compared individually between NiTi coil spring groups and elastomeric chain group, a significant difference in mean RANKL and OPG level were found at 24 h, 168 h, and 1 month after initiation of compressive orthodontic force. Sonis[10] in a comparison study indicated that the elastomeric chain generally lost 50% of the initial applied force in 24 h. The force decay was approximately 35% for 4 days, and they remained relatively constant over the remaining test period. Halimi *et al*.[17] in a review study suggested that the force delivered by the elastomeric chain decays rapidly over time affecting the mechanical properties and clinical efficacy when studied in either human saliva or laboratory test media. Sonis *et al*.[18] in a comparison study between the elastomeric chain and NiTi coil spring concluded that a higher initial force is required to compensate for the high and rapid decay rate for elastomers.

The study also observed that NiTi coil spring has been shown to produce a constant force of varying length, with no decay. van Leeuwen *et al*.[19] in a study to investigate the effect of two different force regimes on tooth displacement concluded that continuous force application was attributed to present a constant responsive state in the cell biology system in opposition to intermittent force application. Taken together, these findings and our present results may suggests that the significant variation in the RANKL and OPG level between NiTi coil spring and elastomeric chain at 24 h, 168 h, and 1 month may be attributed to force decay in elastomeric chain group which provides an intermittent force compared to NiTi coil spring which provides a continuous and constant compressive orthodontic force.

CONCLUSION

- The study suggests that RANKL level increases and OPG level decreases during the application of compressive orthodontic forces
- The study also suggests that the expression of the biomarker of bone resorption (RANKL) and the biomarker of bone formation (OPG) in GCF varies at different time intervals and according to the type of application of force delivery system.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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