Concentration of dentin sialoprotein at the initial stage of orthodontic treatment using self-ligating and conventional preadjusted brackets: A pilot study

Muhammad Sulaiman Kusumah Adiwirya, Retno Widayati, Nurtami Soedarsono[1] and Haru Setyo Anggani

Abstract

OBJECTIVES: This study evaluated differences in concentration of dentin sialoprotein (DSP) in gingival crevicular fluid (GCF) relating to orthodontically induced inflammatory root resorption (OIIRR) at the initial stage of orthodontic treatment using self-ligating and conventional preadjusted brackets.

MATERIALS AND METHODS: Eighteen patients were assigned to three groups of equal size. Two experimental groups received non-extraction orthodontic treatment using passive self-ligating or conventional preadjusted bracket. The control group included patients without orthodontic treatment. GCF was collected from five proximal sites of maxillary anterior teeth at subsequent intervals: immediately prior to orthodontic treatment (T0), and at three and 12 weeks after initiation of treatment (T1 and T2). DSP concentration was evaluated by enzyme-linked immunoabsorbent assay and the differences in DSP levels were analyzed between and within groups.

RESULTS: There were no significant differences in DSP levels within both experimental groups and the control group during T0-T1-T2 ($P \geq 0.05$). A significant difference of DSP concentration was found between the conventional preadjusted bracket and the control group at T2 ($P = 0.038$). However, it was thought to be clinically insignificant.

CONCLUSION: The study showed no significant difference in DSP concentration at the initial stage of orthodontic treatment with either self-ligating or conventional preadjusted bracket.

Keywords:

Conventional, dentin sialoprotein, root resorption, self-ligating

Departments of Orthodontics and [1]Oral Biology, Faculty of Dentistry, Universitas Indonesia, Indonesia

Address for correspondence:
Dr. Muhammad Sulaiman Kusumah Adiwirya, Department of Orthodontics, Faculty of Dentistry, Universitas Indonesia, Jalan Salemba Raya No. 4, Jakarta Pusat, 10430, Jakarta, Indonesia. E-mail: msk.adiwirya@gmail.com

Introduction

Orthodontically induced inflammatory root resorption (OIIRR) is defined as external apical root resorption during orthodontic treatment and is considered an unavoidable pathologic consequence of orthodontic tooth movement. It results in permanent loss of tooth structure from the root apex.[1,2] Maxillary anterior teeth are the most resorbed teeth during orthodontic treatment.[2,3]

Currently, clinical diagnosis of OIIRR is mostly obtained using radiographic techniques.[4] Problems with radiographs usage include that they are technique sensitive and possessing radiation exposure risk, they do not allow identification of the early stages of root resorption, and they only provide two-dimensional information of apical change.[4,5] Histologic studies have

described that OIIRR occurs in 90% of orthodontically treated teeth, while lower estimates of OIIRR were reported with diagnostic radiographic techniques.[6] Therefore, the radiographs may not be adequate diagnostic tool for OIIRR during the first months of orthodontic treatment.[5]

Some studies have investigated the non-invasive alternative method of OIIRR detection at its early stages by using dentin sialoprotein (DSP) in gingival crevicular fluid (GCF) as a suitable biomarker for monitoring root resorption during orthodontic movement.[4,5] DSP is one of the most abundant non-collagenous proteins, beside phosphophoryn (PP), that specifically present within the dentin extracellular matrix.[7] Increasing DSP levels were reported to be detected in GCF after 12 weeks following orthodontic force application.[4]

The etiology of OIIRR is multifactorial, involving genetic predisposition, individual biologic traits, and orthodontic treatment-related clinical risk factors.[2,6] Characteristics that are inseparable from the latter factor include duration of treatment, type and magnitude of the forces applied, the mechanics used, and the type of brackets, are also relevant to the incidence of OIIRR.[8]

Methods of orthodontic treatment mechanics and contemporary appliance designs, have been advanced throughout history of orthodontics to improve the orthodontic treatment efficiency as well as to complement the clinician in achieving the expected result in various orthodontic cases.[9] One such evolution occurred with the use of the self-ligating brackets system, either with an active clip or a passive slide, and this has been gaining popularity in recent years.[8,9] The combination of very low friction and secure archwire engagement is likely to be suggested as the most advantageous feature among any other clinical advantages of such brackets.[10] In addition, self-ligating brackets significantly save appreciable chairside time compared to conventional preadjusted brackets.[8,10] Several studies have investigated the incidence of OIIRR in patients undergoing treatment with self-ligating brackets compared to those undergoing treatment using conventional preadjusted brackets.[11-13] Diagnosis of OIIRR in those studies was mostly carried out by panoramic and periapical radiographs, regardless of their limitation, as mentioned earlier.

The purpose of this prospective study was to compare the concentration of DSP in patients undergoing the initial stage of orthodontic treatment, with self-ligating and conventional preadjusted bracket systems.

Materials and Methods

Ethical approval of the study was obtained from the ethical committee of the Faculty of Dentistry, Universitas Indonesia. In this study, two groups of orthodontic patients together with one group of control subjects were recruited who satisfied the following inclusion criteria: (1) Good general health; (2) healthy periodontal tissue and no sign of gingivitis, with probing depths not exceeding 3 mm; (3) no sign of bone loss in a panoramic radiograph; (4) no active caries; (5) absence of anti-inflammatory drugs in the month prior to the start of the study; (6) no history of antibiotic therapy during the previous six months; (6) moderate score of Little's Irregularity Index[12] on upper anterior teeth; (7) non-extraction orthodontic treatment in the experimental group; and (8) no prior history of orthodontic treatment. One experimental group consisted of six orthodontic patients (three females, three males, mean age 25.5 ± 4.91 years) using self-ligating brackets and the other experimental group consisted of six orthodontic patients (six females, mean age 23 ± 6.28 years) using conventional preadjusted brackets. Six subjects (three females, three males, mean age 26.67 ± 6.83 years) without orthodontic treatment were selected as the control group, which had the same criteria with the orthodontic patients group. All patients who participated in this study gave consent after they had received detailed information about the study protocol.

Experimental design
For this prospective study, subjects were arranged into two experimental groups and one control group [Figure 1]. Group I was treated with passive self-ligating brackets (Damon Q, Ormco, Glendora, CA, USA) and group II was treated with preadjusted brackets (MBT, Ormco, Glendora, CA, USA). Subjects who were not receiving orthodontic treatment were included in the control group. The maxillary arch study models were fabricated using gypsum material (GC America, Chicago, IL, USA) for all subjects in both the experimental and control groups. The Little's Irregularity Index measurement of all maxillary dental casts was taken using digital caliper (ISO13385-1 IP67 ABSOLUTE Coolant-Proof Caliper SERIES 500, Mitutoyo America Corporation, Aurora, IL, USA). This index corresponds to the sum, in millimeters, of the five anatomical contact points from the mesial of the upper right canine to the mesial of the upper left canine.[12] The index measurement and scoring was performed twice with the second measurement repeated by the same experienced examiner after a one-week interval. Another examiner was then assigned to perform the same measurement procedure on the maxillary arch study models to assess the inter-rater reliability.

Both experimental groups were orthodontically treated during the initial leveling and alignment for a 12-week period. Group I had the sequence of 0.014-inch and 0.016-inch copper nickel-titanium archwires (Ormco,
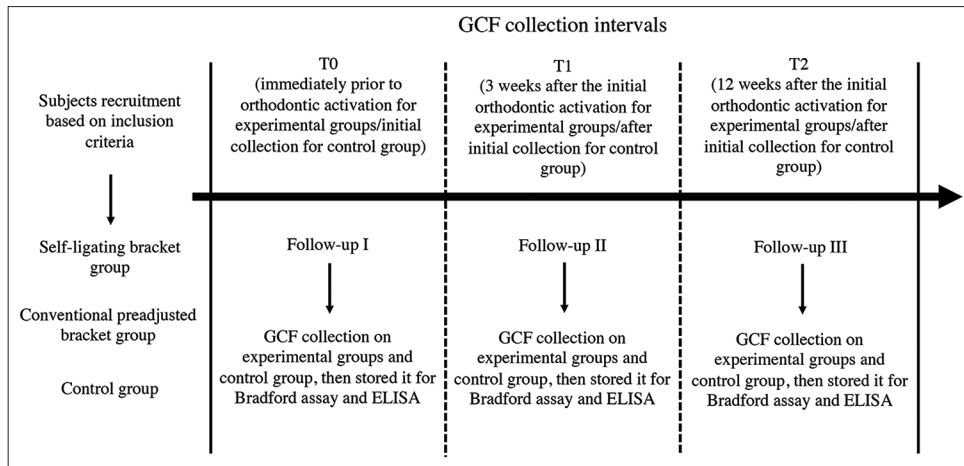
Figure 1: Experimental design

Glendora, CA, USA). Meanwhile, group II had the sequence of 0.014-inch and 0.016-inch nickel-titanium archwires (Dentsply GAC, Bohemia, NY, USA) with elastomeric ligatures (Ormco, Glendora, CA, USA) were used to attach the archwires to the brackets. The six maxillary anterior brackets must be bonded and engaged in the initial orthodontic activation. Bracket debonding or repositioning for both experimental groups was not allowed during the study. All subjects received detailed oral hygiene instructions about the correct use of toothbrush, interdental brush, and dental floss to achieve good plaque control. Moreover, all subjects were informed not to consume any anti-inflammatory drugs throughout study period.

Collection of GCF

The oral hygiene and periodontal health were measured clinically using validated indices at the time of each visit for GCF collection.[14,15] Small deposits of plaque removal were performed using a periodontal probe (Probe 26-G, Medesy, Maniago, PN, Italy) without touching the gingiva to diminish contamination of the GCF samples. GCF was collected immediately prior to orthodontic activation (T0); collection was repeated at three and 12 weeks (T1 and T2) after the initial orthodontic activation for both experimental groups. Collection of GCF for the control group shared the same schedule as the experimental groups, with T0 referred to as the first time GCF was collected, and T1 and T2 referred to as three and 12 weeks after initial GCF collection.

Using the method of Offenbacher *et al*.[16] GCF was collected intrasulcularly at five proximal sites of maxillary anterior teeth, from the mesio-labial side of the right canine through the mesio-labial side of the left canine, and then pooled. The six maxillary anterior teeth were gently cleaned with water, isolated with cotton rolls in the gingival area, and then dried using an air syringe. Insertion of paper points (ISO 30, Gapadent,

Tianjin, PR China) 1 mm into the gingival crevice at each site were carefully performed and allowed to remain for 30 seconds. The procedure was performed twice at each site, with a one-minute interval between collections. Should any paper points visibly contaminated with blood, they will be disposed. Paper points from each GCF collection intervals were then put into a microcentrifuge tube filled with 500 µl of phosphate-buffered saline and kept at -80°C for further processing.

Total protein concentration and DSP determination

The previously stored GCF samples were thawed at room temperature and put to a vortex for 30 seconds. Subsequently, the GCF was completely eluted from the paper points by centrifugal filtration at 1000 x *g* for 15 minutes at 4°C. The separated supernatants resulting from the centrifugation were then transferred into a new microcentrifuge tube. Total protein concentration in the extracts was approximated using the method of Bradford,[17] with bovine serum albumin as standard. The extracts were diluted so as to reach the total protein concentration of 50 µg/ml for a total volume of 450 µl in each tube prior to the sandwich enzyme-linked immunoabsorbent assay (ELISA). DSP concentration in the samples was measured using the Human DSP ELISA Kit (FineTest, Wuhan Fine Biotech Co., Ltd., Wuhan, Hubei, PR China). All samples and reference standards were assayed in duplicate in accordance with the manufacturer's instructions. Measurement of the total DSP concentration present in the GCF samples was obtained from the protein standard curve and then noted as ng/ml.

Statistical methods

Values in this study were reported as the mean ± standard deviation. The data were processed and evaluated using Statistical Package for Social Sciences for Mac (version 21 IBM SPSS Statistics, Chicago, IL, USA). The intra-class correlation coefficient of reliability (ICC) was carried out to

assess intra-rater and inter-rater reliability of irregularity index measurements on the maxillary arch dental casts. Normal distribution of DSP concentrations (ng/ml) was analyzed using Shapiro-Wilk's test and the result showed normal distribution. A two-way repeated measures analysis of variance (ANOVA) with a 95% confidence interval (CI) was used to investigate differences in DSP concentration during experimental periods within all the three groups. Concentrations of DSP were examined with Levene's homogeneity test and the result revealed that all comparison groups had the same variance. The differences in DSP concentration among both orthodontically treated groups and the control group at specific time points were tested by one-way ANOVA (95% CI).

Results

The ICC values for intra-rater and inter-rater evaluation were 0.868 and 0.711, respectively, showing moderate to good agreement with the irregularity index measurement. The mean value of Little's Irregularity Index for the maxillary anterior teeth of self-ligating bracket, conventional preadjusted bracket, and control group were 7.11 ± 1.21 mm, 7.12 ± 1.62 mm, and 6.68 ± 1.06 mm, respectively.

Table 1 shows the changes in DSP concentration for each bracket system and the control subjects during the experimental periods. The control group showed a fluctuation of DSP level through the study, yet it was statistically not significant ($p = 1.00$). The non-significant increase in DSP concentration was also noticed within both orthodontically treated groups during the research time frame ($p > 0.05$). The changes in DSP level for all groups during this study are shown in Figure 2.

Comparisons of the mean DSP values among all the three groups at specific time-points are presented in Table 2. There were no significant differences in DSP concentration between the self-ligating and the conventional preadjusted bracket group at T0, T1, and T2. Even though the conventional preadjusted bracket group showed slightly higher DSP levels than the self-ligating bracket group at the 12-week follow-up, this mean difference did not reach statistical

significance ($p = 0.211$). Interestingly, still at T2, a significant difference in mean DSP concentration was found between the conventional preadjusted bracket group and the control group ($p = 0.038$).

Discussion

OIIRR is a frequent unwanted side effect in orthodontic treatment; thus, contemporary orthodontic techniques and bracket systems have been developed to lessen the problem.[8] Such efforts have been made since OIIRR is considered irreversible once it extends to the dentine. Although this defect can be repaired with cellular cementum covering the remaining dentine, the root never re-establishes back to its original length.[18] The root resorption process itself cannot be separated from the activation of odontoclasts regulated by the RANKL/RANK/OPG system which plays an important role in the orthodontic tooth movement.[19] Odontoclasts are believed to have the same origin progenitor cells as osteoclasts, except they are generally smaller in size, have fewer nuclei, and form smaller resorption lacunae than osteoclasts.[19] Acid phosphatase activity that expressed by the odontoclasts is concluded to be responsible for the resorption of non-collagenous components of the dental organic matrix, including DSP.[20] The dentinal proteins are not considered to be routinely released into periodontal ligament space or even further into the gingival crevice except in the presence of active OIIRR.[5] This process may explain the release of DSP into GCF, which was thought to be related to OIIRR in our study.
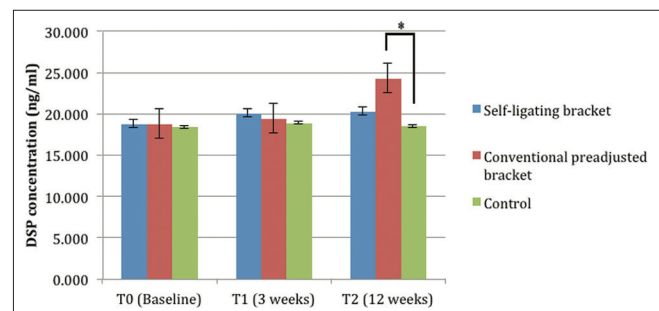

Figure 2: The time-dependent pattern of DSP concentration changes in GCF through 12-weeks follow-up (* *P* < 0.05)

Table 1: DSP concentration longitudinal changes during mechanotherapy in orthodontically treated groups together with control group

Group	n	Mean of DSP concentration (SD) in ng/ml			*p**		
		T0 (*baseline*)	T1 (3-week follow-up)	T2 (12-week follow-up)	T0 vs T1	T0 vs T2	T1 vs T2
Self-ligating bracket	6	18.761±1.560	20.076±2.340	20.299±4.621	0.610	1.000	1.000
Conventional preadjusted bracket	6	18.784±1.945	19.398±3.617	24.315±3.724	1.000	0.060	0.069
Control	6	18.387±0.982	18.872±1.932	18.487±1.734	1.000	1.000	1.000

* Statistically significant difference at *P*<0.05, repeated ANOVA with pairwise comparisons. Bonferroni *post-hoc* test; SD: standard deviation

Table 2: Comparison of DSP concentration changes among orthodontically treated and control group at a specific experimental period

Experimental period	*n*	Mean of DSP concentration (SD) in ng/mL			*p**		
		Self-ligating bracket	Conventional preadjusted bracket	Control	Self-ligating bracket vs conventional preadjusted	Self-ligating bracket vs control	Conventional preadjusted bracket vs control
Baseline	6	18.761(1.560)	18.784 (1.945)	18.387 (0.982)	1.000	1.000	1.000
3-week follow-up	6	20.076(2.340)	19.398 (3.617)	18.872 (1.932)	1.000	1.000	1.000
12-week follow-up	6	20.299(4.621)	24.315 (3.724)	18.487 (1.734)	0.211	1.000	0.038*

*Statistically significant difference at $P<0.05$, one-way ANOVA with Bonferroni *post hoc* test; SD: standard deviation

The definition of the GCF collection intervals in this study has a scientific basis as a small initial resorption lacunae can involve dentin structure as early as three weeks into orthodontic force application.[21,22] Root resorption with tartrate-resistant acid phosphatase (TRAP)-positive multinucleated odontoclasts were also reported to be seen in rats after 21 days subjected to orthodontic forces, especially heavy and jiggling forces.[23] Moreover, DSP levels have been found to rise at 12 weeks following commencement of fixed appliance therapy, and it has been shown that dentin resorption had taken place in that specific time frame.[4,24]

In the current study, the presence of DSP in the control subjects was not anticipated, as their teeth were not undergoing orthodontic force. Parallel to this result, a study demonstrated detection of DSP in untreated control samples without prior history of orthodontic treatment.[4] However, it was assumed that this result was from the basal turnover of dentine matrix proteins which occurs during the maturation process of root structures and which derived from the permanent dentition of younger control subjects that were included in the study.[4] That reason may not suitably explain the circumstance in the present study. Our finding may correlate with an earlier study which discovered the occurrence of apical resorption in 7-10% of samples that had not been treated orthodontically; this was thought to be the result of functional occlusal force or, in other words, physiological root resorption.[25] Some degree of teeth crowding, as exhibited in our control subjects, could also be responsible for the root resorption incidence as it may trigger uneven or excessive exerted pressure on the proximal surfaces.[26] Additionally, there are some suggestions that DSP may not be entirely dentine specific. Qin *et al*.[27] reported DSP detection in extracts of rat long bone at a level of about 1:400 of that in dentine. Another study of rats explored the expression of DSP transcript in osteoblasts of alveolar bone, fibroblasts in periodontal ligament, and cementoblasts in cellular cementum. Even so, the expression level was substantially lower than that of odontoblasts.[28] Despite the fact that those findings may contribute to DSP release into GCF, we found that DSP values in our untreated subjects were

relatively consistent and non-significantly different among intervals, making them still appropriate to be a control group in this study.

Our study showed increasing DSP concentration with a total value of 1.315 ng/ml and 0.614 ng/ml, consecutively, on self-ligating and conventional preadjusted bracket groups during T0-T1. In a similar order, DSP level also increased during T0-T2 as much as 1.538 ng/ml and 5.531 ng/ml. In spite of non-significant values among intervals, this study exhibited increasing DSP levels in a time-dependent pattern within both experimental groups. These present findings further support a study by Kereshanan *et al*.[4] which stated that DSP is discharged into GCF during the initial stages of fixed appliance therapy, although the method of DSP detection was different from the present study. Our current results somewhat also complement an earlier study as elevated levels of DSP could be detected as early as three weeks following orthodontic force application, indicating that resorption had taken place in the dentin structure.[21,22]

The non-significant differences in DSP concentration between the orthodontically treated groups were found during the experimental periods in our study. As Balducci *et al*.[5] suggested that the presence of DSP may reflect the occurrence of OIIRR, we expected a similar degree of dentinal OIIRR to occur in patients treated with both bracket systems. This result is, comparatively, in agreement with previous studies, even though we differed in the duration of orthodontic treatment and the method of diagnosing OIIRR incidence.[11-13]

Another result that required attention from the present study was that the conventional preadjusted bracket group revealed slightly higher DSP levels among the groups at the 12-week follow-up and it was significantly different compared to the control group. We proposed that this phenomenon could happen due to differences of friction value and archwire material, resulting in different net effective forces between the two bracket systems. The use of passive self-ligating brackets in our study was believed to have lower friction value between the archwire and the bracket.[10] Such bracket system has a locking mechanism incorporated into

the bracket that holds the archwire in the bracket slot, instead of using elastomeric ligatures as applied in our conventional preadjusted bracket group producing higher friction.[10,29] It was considered that the addition of copper in copper nickel-titanium archwires used in the self-ligating bracket group would present better defined transition temperatures and reduced hysteresis, ensuring the production of more constant and biologically favorable force to tooth movement.[30,31] Therefore, we presumed that, with the same degree of teeth misalignment among groups in our study, a combination of those two features in the self-ligating bracket system would allow more net optimum force that could be transmitted through the periodontal ligament to the alveolar bone, leading to minimal hyalinization and necrosis of the neighboring tissue, thus reducing the risk of root resorption. However, it was thought to be clinically irrelevant since there was no significant difference of DSP concentration between both bracket systems at 12 weeks following orthodontic activation.

Conclusion

Our present study found no significant difference in DSP concentration with different bracket systems at the initial stage of orthodontic treatment. Patients treated with either the self-ligating bracket or the conventional preadjusted bracket system would probably have a similar chance of OIIRR occurrence. Further long-term clinical studies in a larger number of patients should be carried out to attest the result observed in this research.

Financial support and sponsorship

Conflicts of interest
There are no conflicts of interest.

References

1. Brezniak N, Wasserstein A. Orthodontically induced inflammatory root resorption. Part I: The basic science aspects. Angle Orthod 2002;72:175-9.
2. Topkara A, Karaman AI, Kau CH. Apical root resorption caused by orthodontic forces: A brief review and a long-term observation. Eur J Dent 2012;6:445-53.
3. Sameshima GT, Sinclair PM. Predicting and preventing root resorption: Part I. Diagnostic factors. Am J Orthod Dentofac Orthop 2001;119:505-10.
4. Kereshanan S, Stephenson P, Waddington R. Identification of dentine sialoprotein in gingival crevicular fluid during physiological root resorption and orthodontic tooth movement. Eur J Orthod 2008;30:307-14.
5. Balducci L, Ramachandran A, Hao J, Narayanan K, Evans C, George A. Biological markers for evaluation of root resorption. Arch Oral Biol 2007;52:203-8.
6. Weltman B, Vig KWL, Fields HW, Shanker S, Kaizar EE. Root resorption associated with orthodontic tooth movement: A systematic review. Am J Orthod Dentofac Orthop 2010;137:462-76.
7. Ritchie H. The functional significance of dentin sialoprotein-phosphophoryn and dentin sialoprotein. Int J Oral Sci 2018;10:31.
8. Leite V, Conti AC, Navarro R, Almeida M, Oltramari-Navarro P, Almeida R. Comparison of root resorption between self-ligating and conventional preadjusted brackets using cone beam computed tomography. Angle Orthod 2012;82:1078-82.
9. McLaughlin RP, Bennett JC. Evolution of treatment mechanics and contemporary appliance design in orthodontics: A 40-year perspective. Am J Orthod Dentofac Orthop 2015;147:654-62.
10. Harradine N. Self-ligating brackets increase treatment efficiency. Am J Orthod Dentofac Orthop 2013;143:10-8.
11. Chen W, Haq AAA, Zhou Y. Root resorption of self-ligating and conventional preadjusted brackets in severe anterior crowding Class I patients: A longitudinal retrospective study. BMC Oral Health 2015;15:115-20.
12. Handem RH, Janson G, Matias M, de Freitas KM, de Lima DV, Garib DG, *et al*. External root resorption with the self-ligating Damon system - A retrospective study. Prog Orthod 2016;17:20-5.
13. Qin F, Zhou Y. The influence of bracket type on the external apical root resorption in class I extraction patients - A retrospective study. BMC Oral Health 2019;19:53.
14. Greene JC, Vermillion JR. The simplified oral hygiene index. J Am Dent Assoc 1964;68:7-13.
15. Saxer UP, Mühlemann HR. Motivation and education. SSO Schweiz Monatsschr Zahnheilkd 1975;85:905-19.
16. Offenbacher S, Odle BM, Dyke TEV. The use of crevicular fluid prostaglandin E2 levels as a predictor of periodontal attachment loss. J Periodontal Res 1986;21:101-12.
17. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-54.
18. Feller L, Khammissa RAG, Thomadakis G, Fourie J, Lemmer J. Apical external root resorption and repair in orthodontic tooth movement: Biological events. Bio Med Res Int 2016;2016:4864195.
19. Kamat M, Puranik R, Vanaki S, Kamat S. An insight into the regulatory mechanism of cells involved in resorption of dental hard tissues. J Oral Maxillofac Pathol 2013;17:228-33.
20. Wang Z, McCauley LK. Osteoclasts and odontoclasts: Signaling pathways to development and disease. Oral Dis 2011;17:129-42.
21. Kvam E. Scanning electron microscopy of tissue changes on the pressure surface of human premolars following tooth movement. Scand J Dent Res 1972;80:357-68.
22. Casa MA, Faltin RM, Faltin K, Arana-Chavez VE. Root resorption on torque human premolars shown by tartrate-resistant acid phosphatase histochemistry and transmission electron microscopy. Angle Orthod 2006;76:1015-21.
23. Ueda M, Hikida T, Shimizu M, Kikuta J, Takagi K, Tsukada M, *et al*. Involvement of interleukins-17 and -34 in exacerbated orthodontic root resorption by jiggling force during rat experimental tooth movement. J World Fed Orthod 2020;9:47-55.
24. Weiland F. Constant versus dissipating forces in orthodontics: The effect on initial tooth movement and root resorption. Eur J Orthod 2003;25:335-42.
25. Harris EF, Robinson QC. An analysis of causes of apical root resorption in patients not treated orthodontically. Quintessence Int 1993;24:417-28.
26. Arambawatta K, Peiris R, Ihalagedara D, Abeysundara A, Nanayakkara D. External root resorption of young premolar teeth in dentition with crowding. World J Dent 2013;4:224-7.
27. Qin C, Brunn JC, Cadena E, Ridall A, Tsujigiwa H, Nagatsuka H, *et al*. The expression of dentin sialophosphoprotein gene in bone.

J Dent Res 2002;81:392-4.

28. Baba O, Qin C, Brunn JC, Jones JE, Wygant JN, Mcintyre BW, *et al*. Detection of dentin sialoprotein in rat periodontium. Eur J Oral Sci 2004;112:163-70.

29. Baccetti T, Franchi L, Camporesi M, Defraia E, Barbato E. Force produced by different nonconventional bracket or ligature systems during alignment of apically displaced teeth. Angle Orthod 2009;79:533-9.

30. Higa RH, Semenara NT, Henriques JFC, Janson G, Sathier R, Fernandes TMF. Evaluation of released by deflection of orthodontic wires in conventional and self-ligating brackets. Dental Press J Orthod 2016;6:91-7.

31. Gravina MA, Brunharo IHVP, Canavarro C, Elias CN, Quintao CCA. Mechanical properties of NiTi and CuNiTi shape-memory wires used in orthodontic treatment. Part 1: Stress-strain tests. Dental Press J Orthod 2013;18:35-42.