Original Article

Role of six cytokines and bone metabolism biomarkers in gingival crevicular fluid in patients undergoing fixed orthodontic appliance treatment in comparison with aligners: a clinical study

Muhammad Abdullah Kamran[a]; Abdullah A. Alnazeh[a]; Mohammad Almagbol[b]; Salem Almoammar[a]; Ali Hasan A. Alhaizaey[c]; Ibrahim Alshahrani[d]

ABSTRACT

Objectives: The objective of this study was to assess bone biomarkers and cytokines in patients with conventional labial appliances (CLAs) and aligners.

Materials and Methods: Participants were recruited to undergo orthodontic treatment with CLAs and aligners according to predefined inclusion and exclusion criteria. Periodontal examination was accomplished at baseline and 4 weeks using the plaque index (PI), gingival index (GI), and bleeding on probing (BoP). Samples of gingival crevicular fluid (GCF) were collected at baseline (T0) before the start of treatment and at the 1-month follow-up (T1) to assess bone metabolic and inflammatory biomarkers. GCF from participants with CLAs and aligners was evaluated with enzyme-linked immunosorbent assay. Comparison between labial conventional orthodontic treatment and aligners were assessed using an unpaired *t*-test. The difference between T0 and T1 was measured using a paired *t*-test.

Results: BoP, PI, and GI demonstrated no significant difference between participants treated with aligners and subjects with CLAs at baseline and at 4 weeks ($P > .05$). Bone markers and other biomarkers (tumor necrosis factor α, interleukin [IL]-α, IL-2, IL-6, and IL-8) showed significant differences ($P < .05$). Also, a significant difference between CLAs and aligners was noted among all biomarkers ($P < .05$) except IL-β.

Conclusions: Aligners and CLAs increase the level of inflammatory and bone metabolic biomarkers after 1 month. (*Angle Orthod.* 2023;93:335–340.)

KEY WORDS: Conventional appliance; Gingival crevicular fluid; Aligner; Bone metabolism biomarker; Inflammatory biomarkers

INTRODUCTION

The mechanical stimulus due to orthodontic force applied should be continuous and smooth as it promotes cell and tissue changes in the periodontium, leading to the modeling and remodeling of alveolar bone.[1] This is followed by the release of inflammatory cytokines that modifies the connective tissue and encourages activation of osteoclastic activity.[2] Gingival crevicular fluid (GCF) is an inflammatory exudate and is used to sense bone-remodeling molecules along with various cytokines, electrolytes, antigens, and proteins.[3–5]

On the application of orthodontic force and plaque accumulation, an inflammation of periodontal tissues is observed. The inflammatory response results in the activation of cytokines.[5] These cytokines may range from matrix metalloproteins to interleukin (IL) and tumor necrosis factor α (TNF-α).[5,6] In addition, bio-

[a] Associate Professor, Department of Pedodontics and Orthodontic Sciences, College of Dentistry, King Khalid University, Abha, Saudi Arabia.

[b] Assistant Professor, Department of Community Dentistry and Periodontics, Faculty of Dentistry, King Khalid University, Abha, Saudi Arabia.

[c] Assistant Professor, Department of Orthodontics, King Faisal Medical City, Abha, Saudi Arabia.

[d] Professor Department of Pedodontics and Orthodontic Sciences, College of Dentistry, King Khalid University, Abha, Saudi Arabia

Corresponding author: Dr Muhammad Abdullah Kamran, Associate Professor, Department of Pedodontics and Orthodontic Sciences, College of Dentistry, King Khalid University, Postal code 61421, Abha, Saudi Arabia
(e-mail: mmuhammad@kku.edu.sa)

Accepted: December 2022. Submitted: September 2022.
Published Online: February 20, 2023

chemical markers of bone metabolism, such as osteoprotegerin (OPG) and osteopontin (OPN), and different transforming growth factors that result in bone modeling and remodeling also get triggered.[2,7]

Due to the awareness and appeasement of esthetic demands, orthodontic aligners are suggested.[8,9] Despite their spread and usage among the community of orthodontics, there is very limited evidence of bone metabolism induced by them.[9,10] Aligners provide intermittent orthodontic forces that alter the typical stages of tooth movement as previously discussed by Krishnan and Davidovitch.[1,11] However, compared with fixed orthodontic appliances, aligners facilitate good oral hygiene along with esthetics and patient comfort.[8,12] However, they have a disadvantage in treating specific types of malocclusion and have a higher cost.

To date, there is little evidence of the production of biochemical markers of bone metabolism and other inflammatory mediators in patients undergoing labial fixed appliances compared with aligners. It was hypothesized that there would be no difference in bone biomarkers and cytokine levels between participants going through treatment with fixed labial orthodontic appliances and aligners. Therefore, the present study aimed to assess the level of different bone biomarkers and levels of cytokines in patients with conventional fixed labial appliances and aligners at baseline (T0) and at the 1-month follow-up (T1).

MATERIALS AND METHODS

Study Design and Participants

Participants were patients recruited from the orthodontic department of King Khalid University undergoing orthodontic treatment with labial conventional fixed appliances and aligners. The ethical board of King Khalid University approved the study. The study was in compliance with the Declaration of Helsinki. Written and verbal consent was obtained from participants before the start of the study. To minimize treatment bias, only participants with mild malocclusion with between 2 mm and 4 mm of crowding were included. Patients were randomly allocated into two groups, that is, patients treated with aligners and patients treated with the fixed labial appliances. For sample size calculation, 11 participants were found to be enough in each group, assuming a level of significance of .05 and a study power of 0.80 to perceive a significant difference. However, due to the underestimation of power, potential sample loss, and the dropout for follow-up, a sample of 25 participants in each group was determined to be enough (50 total participants). The mean age of the participants in each group was 25 $\pm$ 4 years (range, 15 to 30 years) with a male-to-female ratio of participants treated with aligners (15 males/10 females) and participants treated with conventional fixed appliances (17 males/8 females).

Participants met the following inclusion criteria: (1) healthy systemic condition, (2) no use of anti-inflammatory drugs or antibiotics in the past 6 months, (3) minor malocclusion (2 to 4 mm of crowding), (4) periodontal parameters of gingival index <1 and no pocketing with a generalized pocketing depth of ≤ 3, (5) radiographic parameters of no crestal bone loss, (6) no history of smoking, and (7) extraction of third molars. Participants were excluded using the following criteria: (1) teeth missing; (2) systemic conditions (kidney disease, HIV, and liver disease); or (3) poor oral hygiene, signs of inflammation of the gingiva, and periodontitis. For patients who were aged younger than 18 years, informed consent was obtained from the parent/guardian. Initially, before the start of the trial, all participants were given oral hygiene instructions, that is, education on brushing techniques, use of fluoridated water and toothpaste, and frequency of brushing. Two weeks before the study, prophylactic supra and subgingival scaling were performed. The participants were kept motivated throughout the study through periodic follow-up.

Examination of Periodontal Parameters

A periodontal examination was performed at baseline using the plaque index (PI), gingival index (GI), and bleeding on probing (BoP). The teeth assessed for PI in the upper arch were the maxillary right first molar and lateral incisor and maxillary left first premolar. Similarly, in the lower quadrant, the teeth assessed were the mandibular left first molar and lateral incisor and mandibular right first premolar. GI was obtained at four sites of teeth and were measured and scored labial, lingual, distal, and mesiolingual. The same technique was repeated after 4 weeks.

Measuring and Collecting GCF

Samples of GCF were collected at T0 before the start of treatment and T1. Collection of GCF was done after 4 weeks as indirect resorption occurs after 28 days.[13,14] The area was selected where gingival inflammation was found to be minimal, and the site for sample collection was kept homogenious for all participants (proximal to canines in the upper arch). Isolation was assured using sterilize gauze to minimize contamination. GCF was collected by placing the pipette at the sulcus of the gingiva, mildly touching the margin of the gingiva.[15,16] An effort was made to collect 1 μL of GCF with the pipette, adopting the extracrevicular approach. Pipettes contaminated with blood or saliva during the procedure were excluded. The collected GCF was transferred to 0.5 mL Eppendorf tubes, centrifuged at 3000 rpm, and stored

Table 1. Periodontal Parameters at Baseline and at the 1-Month Follow-Up in Participants Undergoing Orthodontic Treatment With CLAs and Aligners[a]

	Baseline (n = 25 Each)		1-Month Follow-Up (n = 25 Each)		
Periodontal Parameters	CLA	Aligner	CLA	Aligner	*P* Value[b]
BoP, mean (range)	0.77 (0.2–1.1)	0.51 (0.1–0.7)	0.81 (0.3–0.9)	0.49 (0.1–0.6)	**.33**
GI, mean (range)	0.4 (0.1–0.7)	0.3 (0.09–0.5)	0.6 (0.1–0.8)	0.4 (0.09–0.6)	**.55**
PI, mean (range)	0.86 (0.3–1.1)	0.71 (0.41–1.3)	0.91 (0.4–1.1)	0.89 (0.44–1.2)	**.25**

[a] BoP indicates bleeding on probing; CLA, conventional labial appliance; GI, gingival index; and PI, periodontal index.
[b] Bold denotes significance.

at –80°C for 10 minutes until the time of assay. Blinding was maintained throughout the process.

Analysis of Cytokines Using Enzyme-Linked Immunosorbent Assay

The frozen samples of GCF from participants with both labial conventional orthodontic appliances and aligners were thawed at room temperature for enzyme-linked immunosorbent assay (ELISA). The biologist in the biochemistry laboratory at King Khalid University, who was blinded, performed the cytokine measurement (ie, $\kappa = 0.71$). Bone metabolism biomarkers IL-1β, receptor activator of nuclear factor kappa-ligand (RANK-L), OPG, and OPN and other inflammatory biomarkers TNF-α, IL-1α, IL-2, IL-6, and IL-8 were assessed by ELISA, as recommended by the manufacturer. In short, samples and standards were combined into the matching wells. Incubation was done overnight at 4°C. Inclusion of 0.1 mL of 1% biotinylated anti-human C-reactive protein detector antibody to individual wells was performed. The incubation was done at a room temperature of 25°C for 60 minutes in the dark.[17,18] Before incubating, 0.1 mL of 1% horseradish peroxidase–streptavidin solution was incorporated into each well for 45 minutes at 25°C. The sensitivity of ELISA for all GCF cytokines was above 99.1%.

Statistical Analysis

For analysis, statistical software (SPSS version 18, IBM, Chicago, Ill) was used. Normality of data was assessed using the Kolmogorov-Smirnov test. The levels of cytokines were displayed as means. Comparison between labial conventional orthodontic appliance treatment and aligners was assessed using an unpaired *t*-test. Similarly, the difference between T0 and T1 was measured using a paired *t*-test. The level of significance was set at $P = .05$.

RESULTS

Periodontal parameters, that is, BoP, PI, and GI, demonstrated no significant difference between participants treated with aligners and participants treated with conventional labial appliances (CLAs) at T0 and T1 ($P > .05$) (Table 1).

The levels of TNF-α and IL-β in the GCF of participants treated with CLAs were increased compared with participants treated with aligners. Similarly, IL-2, IL-6, and IL-8 demonstrated high levels in patients treated with aligners compared with CLAs. Among bone markers, OPN and RANK-L in the GCF demonstrated an upsurge in CLA participants compared with aligners. Similarly, the level of OPG showed a descent from baseline to follow-up in both experimental groups. The level of bone markers and the biomarkers TNF-α, IL-α, IL-2, IL-6, and IL-8 showed significant differences from baseline to follow-up ($P > .05$). Also, a significant difference between CLAs and aligners was noted in all biomarkers ($P < .05$) except IL-β ($P < .05$) (Table 2).

Table 2. Levels of Cytokines and Bone Metabolism Biomarkers in GCF (pg/mL) of Patients Undergoing Fixed Orthodontic Treatment With CLAs and Aligners (n = 25 each) at T0 and T1[a]

Biomarkers in GCF	Groups	T0, Mean ± SD	T1, Mean ± SD	*P* Value
TNF-α (pg/mL)	CLA	7.22 ± 1.21	14.21 ± 1.21[b]	.01[c]
	Aligner	5.25 ± 1.65	11.28 ± 1.22[b]	.01[c]
IL-α (pg/mL)	CLA	0.082 ± 0.001	6.68 ± 1.22[b]	.02[c]
	Aligner	0.025 ± 0.004	9.22 ± 1.55[b]	.01[c]
IL-β (pg/mL)	CLA	8.90 ± 0.22	21.45 ± 1.88	.01[c]
	Aligner	7.94 ± 0.66	18.29 ± 2.57	.01[c]
IL-2 (pg/mL)	CLA	3.9 ± 1.23	13.9 ± 2.05[b]	.01[c]
	Aligner	2.98 ± 1.01	16.3 ± 2.14[b]	.01[c]
IL-6 (pg/mL)	CLA	3.65 ± 1.21	12.25 ± 1.26[b]	.02[c]
	Aligner	3.79 ± 1.82	17.88 ± 1.58[b]	.01[c]
IL-8 (pg/mL)	CLA	0.68 ± 0.02	1.39 ± 0.84[b]	.01[c]
	Aligner	0.74 ± 0.04	3.91 ± 0.11[b]	.01[c]
OPG (pg/μL)	CLA	51.171 ± 13.54	19.23 ± 12.33[b]	.01[c]
	Aligner	49.25 ± 14.33	23.75 ± 11.11[b]	.01[c]
OPN (ng/μL)	CLA	11.25 ± 1.56	23.85 ± 1.41[b]	.01[c]
	Aligner	10.89 ± 1.89	17.10 ± 0.85[b]	.01[c]
RANK-L (pg/μL)	CLA	0.7 ± 0.1	1.9 ± 0.2[b]	.01[c]
	Aligner	0.6 ± 0.1	1.3 ± 0.1[b]	.01[c]

[a] CLA, conventional labial appliance; GCF, gingival crevicular fluid; IL, interleukin; OPG, osteoprotegerin; OPN, osteopontin; RANK-L (receptor activator of nuclear factor kappa-ligand); SD, standard deviation; T0, baseline; T1, at the 1-month follow-up; TNF-α, tumor necrosis factor α.
[b] Labial appliances and aligners compared with unpaired *t*-tests showing statistical significance ($P < .05$).
[c] Denotes statistically significant changes in levels of cytokines from baseline to follow-up within groups by paired *t*-tests.

DISCUSSION

The present study was based on the null hypothesis that there would be no difference in bone biomarkers and cytokine levels between participants undergoing treatment with fixed labial orthodontic appliances and aligners.[19,20] The force with aligners and CLAs triggered an inflammatory response in periodontal tissues, activating chemokines, cytokines, proteolytic enzymes, and prostaglandins and bone metabolism biomarkers associated with alveolar apposition.[19,21] In the present study, the gingival sulcus was selected as the site of testing as the force was applied to the teeth due to ease of access and because GCF is known to be a consistent tool to measure variations in the disease processes.[22,23]

Bone biomarkers, along with different cytokines, are responsible for bone resorption and bone formation activities. IL-2, IL-6, IL-8, and TNF-α are considered to be proinflammatory cytokines,[24,25] whereas IL-4, IL-10, and IL-13 are considered to be anti-inflammatory.[26,27] IL-6 is related to osteoclastic activity and resorbs bone. Various studies have shown that IL-6 increases in the first few weeks when force is applied with no upsurge.[28,29] This was observed in the present study in both experimental groups treated with CLAs and aligners from baseline to follow-up. Similarly, IL-8 was found to be higher in participants with aligners rather than in the CLA group at follow-up. IL-8 is secreted by various cell monocytes, endothelial cells, and fibroblasts as a response to TNF-α and IL-1.[30,31] Recent work by Basaran et al. found that orthodontic forces at sites of tension evoked a cascade of the proinflammatory cytokines IL-2, IL-6, IL-1, and TNF-α.[13,32] The authors speculated that, with aligners, the forces were intermittent, which caused a greater increase in IL-8 levels among the participants in the aligner therapy groups. Among the inflammatory cytokines, TNF-α, IL-1, and IL-2 have a primary role in the initiation of the cascade, proinflammatory response, and pathogenesis of disease.[33,34] At the cellular level, TNF-α and IL-2 are responsible for the induction of the other mediators IL-6, IL-8, matrix metalloproteinase, and prostaglandins.[35] The work by Basaran et al. indicated that TNF-α, IL-2, and IL-β, which is a physiological part of IL-2, showed an upsurge during leveling and distalization.[13] Similarly, a recent study by Castroflorio et al. observed that the level of IL-β was increased on the tension side.[36] Also, evidence from the study by Vujacic et al. showed that mechanical stress due to orthodontic force increased the levels of IL-β and TNF-α.[37] The findings of the present study were consistent with previous studies.

Alveolar remodeling is controlled by an equilibrium between RANK-L binding and the production of OPG. For activation and differentiation of osteoclasts, the RANK-L signaling pathway is crucial.[38] OPG is expressed in both RANK-L and osteoblastic cells, and it is a decoy receptor that competes with receptor activator of nuclear factor kappa (RANK) for the binding of RANK-L. An increase in RANK-L and a decrease in OPG indicate compressive force.[38] The findings of the present study were in agreement with this argument as, in the current study, the level of OPG decreased at follow-up compared with baseline, but there was a climb in RANK-L noted after 4 weeks. However, work by Kobayashi et al. stated that RANK-L may have a short duration (within 1 hour, 24 hours of orthodontic force application) and role in bone changes.[38] The present study was in disagreement with this finding as the level of RANK-L was found to be increased after 4 weeks as well.

OPN is a bone protein, noncollagenous in nature, and triggers osteoblastic activity during an early stage of cell differentiation, helps in biomineralization, mediates the control of RANK-L expression, and has the property to inhibit osteoclastic activity.[36] The levels of OPN increase when mechanical orthodontic force is applied and at tension sites.[36] This is consistent with the present study.

When evaluating periodontal parameters, no significant difference was noted in participants treated with CLAs and aligners. Although BoP, GI, and PI were somewhat lower in aligner-treated participants, the difference compared with fixed appliance patients was not statistically significant. Periodontal status was found in previous studies to be better in participants treated with aligners, as there is evidence that aligners facilitated better oral hygiene and compliance and minimize the growth of periodontal pathogens.[9,12] However, in the current study, because no significant changes were reported in periodontal parameters, this indicated that levels of cytokines and bone biomarkers were within the limit of acceptable physiological response.

Within the limitations of the present study, measuring tooth movement with CLAs and aligners may help to correlate the inflammatory biomarker response in a more conclusive manner. Individuals respond differently to mechanical loading. Age, sex, and density of bone are variable and may contribute differently. The short duration of the current study can be considered a major limitation. More clinical trials along with a split-mouth design are recommended to extrapolate the findings of the present study.

CONCLUSION

- An orthodontic force applied with aligners and CLAs surges inflammatory (TNF-α, IL-2, IL-6, IL-8, IL-α, IL-β) and bone metabolic biomarkers (OPN, OPG,

RANK-L) after 1 month of follow-up. However, some cytokines increased more than others.

ACKNOWLEDGMENTS

The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University for funding this work through the Small Groups Project under grant number RGP.1/198/43.

REFERENCES

1. Krishnan V, Davidovitch Z. Cellular, molecular, and tissue-level reactions to orthodontic force. *Am J Orthod Dentofacial Orthop*. 2006;129:469.e1–469.e32.
2. Alhadlaq AM, Patil S. Biomarkers of orthodontic tooth movement in gingival crevicular fluid: a systematic review. *J Contemp Dent Pract*. 2015;16:578–587.
3. Rody WJ, Wijegunasinghe M, Wiltshire WA, Dufault B. Differences in the gingival crevicular fluid composition between adults and adolescents undergoing orthodontic treatment. *Angle Orthod*. 2014;84:120–126.
4. Ito H, Numabe Y, Hashimoto S, et al. Correlation between gingival crevicular fluid hemoglobin content and periodontal clinical parameters. *J Periodontol*. 2016;87:1314–1319.
5. Hancock EB, Cray RJ, O'Leary TJ. The relationship between gingival crevicular fluid and gingival inflammation: a clinical and histologic study. *J Periodontol*. 1979;50:13–19.
6. Gupta G. Gingival crevicular fluid as a periodontal diagnostic indicator—I: host derived enzymes and tissue breakdown products. *J Med Life*. 2012;5:390–397.
7. Wassall RR, Preshaw PM. Clinical and technical considerations in the analysis of gingival crevicular fluid. *Periodontol 2000*. 2016;70:65–79.
8. Rossini G, Parrini S, Castroflorio T, Deregibus A, Debernardi CL. Periodontal health during clear aligners treatment: a systematic review. *Eur J Orthod*. 2015;37:539–543.
9. Karkhanechi M, Chow D, Sipkin J, et al. Periodontal status of adult patients treated with fixed buccal appliances and removable aligners over one year of active orthodontic therapy. *Angle Orthod*. 2013;83:146–151.
10. Pithon MM, Baião FCS, de Andrade Sant Anna LID, Paranhos LR, Cople Maia L. Assessment of the effectiveness of invisible aligners compared with conventional appliance in aesthetic and functional orthodontic treatment: a systematic review. *J Investig Clin Dent*. 2019;10:e12455.
11. Nakao K, Goto T, Gunjigake KK, Konoo T, Kobayashi S, Yamaguchi K. Intermittent force induces high RANKL expression in human periodontal ligament cells. *J Dent Res*. 2007;86:623–628.
12. Levrini L, Novara F, Margherini S, Tenconi C, Raspanti M. Scanning electron microscopy analysis of the growth of dental plaque on the surfaces of removable orthodontic aligners after the use of different cleaning methods. *Clin Cosmet Investig Dent*. 2015;7:125–131.
13. Başaran G, Özer T, Kaya FA, Hamamci O. Interleukins 2, 6, and 8 levels in human gingival sulcus during orthodontic treatment. *Am J Orthod Dentofacial Orthop*. 2006;130:7.e1–7.e6.
14. Drummond S, Canavarro C, Perinetti G, Teles R, Capelli J. The monitoring of gingival crevicular fluid volume during orthodontic treatment: a longitudinal randomized split-mouth study. *Eur J Orthod*. 2012;34:109–113.
15. Jamesha FI, Maradi AP, Chithresan K, Janakiram S, Maddur PK, Rangaraju R. Comparison of gingival crevicular fluid periostin levels in healthy, chronic periodontitis, and aggressive periodontitis. *J Indian Soc Periodontol*. 2018;22:480–486.
16. Aral CA, Köseoğlu S, Sağlam M, Pekbağrıyanık T, Savran L. Gingival crevicular fluid and salivary periostin levels in non-smoker subjects with chronic and aggressive periodontitis: periostin levels in chronic and aggressive periodontitis. *Inflammation*. 2016;39:986–993.
17. Alasqah MN. Influence of adjunctive non-surgical peri-implant therapy on clinical and salivary cytokine profile in obese patients. *Photodiagnosis Photodyn Ther.* 2022;37. 102721–102725.
18. Alqutub MN. Peri-implant parameters and cytokine profile among peri-implant disease patients treated with Er Cr YSGG laser and PDT. *Photodiagnosis Photodyn Ther.* 2022; 37. 102641–102645.
19. Jiang Q, Li J, Mei L, et al. Periodontal health during orthodontic treatment with clear aligners and fixed appliances: a meta-analysis. *J Am Dent Assoc*. 2018;149:712–720.e12.
20. Rossini G, Parrini S, Castroflorio T, Deregibus A, Debernardi CL. Periodontal health during clear aligners treatment: a systematic review. *Eur J Orthod*. 2015;37:539–543.
21. Kaygisiz E, Uzuner FD, Yuksel S, et al. Effects of self-ligating and conventional brackets on halitosis and periodontal conditions. *Angle Orthod*. 2015;85:468–473.
22. Kanshin E, Wang S, Ashmarina L, et al. The stoichiometry of protein phosphorylation in adipocyte lipid droplets: Analysis by N–terminal isotope tagging and enzymatic dephosphorylation. *Proteomics*. 2009;9:5067–5077.
23. Sueda T, Bang J, Cimasoni G. Collection of gingival fluid for quantitative analysis. *J Dent Res*. 1969;48:159.
24. Hamamci N, Acun Kaya F, Uysal E, Yokuş B. Identification of interleukin 2, 6, and 8 levels around miniscrews during orthodontic tooth movement. *Eur J Orthod*. 2012;34:357–361.
25. Jayaprakash P, Basavanna J, Grewal H, Modi P, Sapawat P, Bohara P. Elevated levels of interleukin (IL)-1β, IL-6, tumor necrosis factor-α, epidermal growth factor, and β2-microglobulin levels in gingival crevicular fluid during human orthodontic tooth movement (OTM). *J Fam Med Prim Care*. 2019;8:1602.
26. Al-Ghurabi BH, Mohammed-Salih HS, Ghazi A, Saloom HF. Evaluation of salivary levels of proinflammatory cytokines (IL-1α, IL-8 and GM-CSF) in adult orthodontic patients. *J Dent Med Sci*. 2014;13:75–78.
27. Rody WJ, Wijegunasinghe M, Wiltshire WA, Dufault B. Differences in the gingival crevicular fluid composition between adults and adolescents undergoing orthodontic treatment. *Angle Orthod*. 2014;84:120–126.
28. Mohammed A, Saidath K, Mohindroo A, et al. Assessment and measurement of interleukin 6 in periodontal ligament tissues during orthodontic tooth movement. *World J Dent*. 2019;10:88–92.
29. Kunii R, Yamaguchi M, Tanimoto Y, et al. Role of interleukin-6 in orthodontically induced inflammatory root resorption in humans. *Korean J Orthod*. 2013;43:294–301.
30. Hamamci N, Acun Kaya F, Uysal E, Yokuş B. Identification of interleukin 2, 6, and 8 levels around miniscrews during

orthodontic tooth movement. *Eur J Orthod*. 2012;34:357–361.
31. Baggiolini M, Walz A, Kunkel SL. Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. *J Clin Invest*. 1989;84:1045–1049.
32. Drummond S, Canavarro C, Perinetti G, Teles R, Capelli J. The monitoring of gingival crevicular fluid volume during orthodontic treatment: a longitudinal randomized split-mouth study. *Eur J Orthod*. 2012;34:109–113.
33. Perinetti G, Primožič J, Castaldo A, Di Lenarda R, Contardo L. Is gingival crevicular fluid volume sensitive to orthodontic tooth movement? A systematic review of split-mouth longitudinal studies. *Orthod Craniofacial Res*. 2013;16:1–19.
34. Batra P, Kharbanda OP, Duggal R, Singh N, Parkash H. Alkaline phosphatase activity in gingival crevicular fluid during canine retraction. *Orthod Craniofacial Res*. 2006;9: 44–51.
35. Karaduman B, Uraz A, Altan GN, et al. Changes of tumor necrosis factor-α, interleukin-10, and tartrate-resistant acid phosphatase5b in the crevicular fluid in relation to orthodontic movement. *Eur J Inflamm*. 2015;13:3–13.
36. Castroflorio T, Gamerro EF, Caviglia GP, Deregibus A. Biochemical markers of bone metabolism during early orthodontic tooth movement with aligners. *Angle Orthod*. 2017;87:74–81.
37. Ren Y, Vissink A. Cytokines in crevicular fluid and orthodontic tooth movement. *European journal of oral sciences*. 2008;116:89–97.
38. Kobayashi Y, Hashimoto F, Miyamoto H, et al. Force-induced osteoclast apoptosis in vivo is accompanied by elevation in transforming growth factor β and osteoprotegerin expression. *J Bone Miner Res*. 2000;15:1924–1934.