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Original Article

A Comparative Study on the IL-8 Expression in Gingival Crevicular Fluid during Early Alignment Stage of Orthodontic Treatment in Adults and Adolescents

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ABSTRACT

Background: Orthodontic tooth movement causes the release of various biomolecules such as interleukins. The aim of this study was to compare IL-8 expression in gingival crevicular fluid during early alignment stage of orthodontic treatment in adults and adolescents.

Methods: The present study was done on 20 orthodontic patients, including 10 adolescents and 10 adults. Before bonding, gingival crevicular fluid (GCF) was collected with a paper strip in gingival sulcus of maxillary right central incisor on the distolabial aspect for 60 seconds, followed by bonding and insertion of initial 14 NiTi arch wire. GCF collection was repeated 24 hours, 7 days, and 28 days after bonding. The IL-8 levels was measured by the enzyme-linked immunosorbent assay (ELISA). Data were analyzed by SPSS version 21 using repeated measurement test. Statistically significant level was considered at P = 0.05.

Results: In the present study, 14 patients (70%) were female and the rest were male. The mean age of the patients was 19.11 ± 6.23 years. The level of IL-8 at the baseline was higher than the other time periods, and on the first day after treatment, significantly decreased compared to the baseline. There was no statistically significant difference between age groups <19 years and ≥19, and also, gender in different time periods in terms of IL-8 level.

Conclusion: The results of the present study revealed that the level of IL-8 significantly decreased on the first day of orthodontic treatment, and then, increased. There was no statistically significant difference between gender and age and IL-8 levels during treatment. Further studies with larger sample sizes and different treatment methods are recommended.

Keywords: Cytokine, IL-8, Tooth movement, Orthodontic treatment, Gingival crevicular fluid

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Introduction

The orthodontic movement of teeth depends on the harmonic analysis and forming the tissues around the bone and periodontal ligament (1). The initial phase of orthodontic tooth movement (OTM) begins with an acute inflammatory response, which is characterized by vasodilation and increased vascular permeability. Orthodontic forces cause cellular and vascular changes in the periodontal ligament and the movement of extracellular fluid in the ligament and pressure and tension of collagen fibers and extracellular matrix (1). This process leads to the release of various biomolecules including interleukin, TNF alpha (TNF-α), macrophage colony-stimulating factor, enzymes, pro-inflammation mediators such as prostaglandin, growth factor, and vasoactive neurotransmitters (3, 4). Some pro-inflammation cytokines, such as IL-1B, IL-6, IL-8, and TNF-α, play a major role as a regulator of bone remodeling due to mechanical stimulations (5). Cytokines are secreted by leukocytes and may interact directly with osteoclasts or indirectly through monocytes, macrophages, lymphocytes, and fibroblasts via the production of cytokines or other growth factors (6, 7). Molecular events commence the bone remodeling process and result in effective tooth movement. Biomechanical mediators, released at various stages in dental movement, can be followed in the gingival crevicular fluid (GCF) (8). The use of GCF analysis in monitoring the biological response during orthodontic treatment is a good choice due to its non-invasive nature, uncomplicated and feasibility of collecting fluid at different time periods (9, 10).

Yang et al. (2013) showed that orthodontic forces increased the expression of interleukins 6 and 8 (11). Giannopoulou et al. (2008) reported an increase in the level of interleukin-8 during orthodontic tooth movement (12). Gujar et al. (2019) showed that the levels of cytokines (IL-1α, IL-1ß, IL-2, IL-6, IL-8, and TNF-α) increased in the GCF three weeks after conventional treatment with labial fixed appliance (13). Biological understanding of cytokines as a response to forces applied to orthodontic tooth movement is difficult due to complexity and variety of these cytokines (8). It has been shown that the rate of bone turnover decreases in adult patients due to the limited number of progenitor cells (14, 15), and their ability to form blood vessels (16, 17), and also, decreased the density of fibroblasts (18). Thus, it is supposed that a decrease in response in the older dentoalveolar complex causes problems in bone formation and resorption during orthodontic tooth movement (15, 19). The time of treatment is longer in adults and the onset of tooth movement is later than that in adolescents (20). The present study aimed to determine the level of IL8 in GCF during the initial stage of orthodontic treatment leveling in adolescent and adult patients in Kerman.

Methods and Materials

This descriptive-analytical study was conducted on 20 orthodontic patients who referred to the specialized orthodontic centers in Kerman. The patients included 10 adolescent patients under the age of 19 years and 10 adult patients over the age of 19 years. The inclusion criteria of the study included:

1. Good general health
2. Not taking antibiotics in the last 6 months
3. Not taking anti-inflammatory drugs in the last month
4. The absence of periodontal disease (probing depth in all teeth ≤ 3 mm, with no attachment loss and no radiographic evidence of bone resorption)
5. Class I malocclusion
6. Incisor irregularity index between 3 to 6 mm
7. Having permanent dentition
8. No need to tooth extraction in the treatment plan
9. Willingness to participate in the study

After selecting the patients, first, the aim of the study was explained to them and after obtaining their written consent (consent of parents for patients under the age of 18 years), they were included into the study. Information about age and gender was recorded in a checklist. One week before bonding, scaling was performed for all patients, and then, oral health education were provided for them. Before orthodontic treatment and after drying the patients' tooth surface with air spray and isolation with a cotton roll, GCF was collected before any other treatment.

Paper tape was placed in the gingival sulcus 1-2 mm below the gingival margin in the distolabial site of the maxillary central teeth for 60 seconds. Then, fixed orthodontic treatment was done using MBT 0.022-inch slot bracket system with placing the initial Archwire 14 TiNi (OrthoTechnology). The patient was called and gingival crevicular fluid was collected 24 hours,

7 days, and 28 days after the beginning of treatment (3, 21, 22). Then, the paper tape was placed in a sterile tube, sent to the laboratory, and kept in a refrigerator at -20°C until the experiment day. It should be noted that the paper tape contaminated with saliva or blood were excluded from the study. Gingival fluid collection for patients' matching was performed in all patients in the evening between 4 p.m. and 5 p.m. In this study, IL-8 was measured using enzyme-linked immunosorbent assay (ELISA).

The measurement was performed using ab46052 kit (England). The sample preparation method was performed according to the manufacturers' instructions. The concentrations of the samples prepared with standard IL-8 were compared. The concentration of each biomarker (pg/µL) was measured using bioTek (ELx 808, the USA). The obtained data were entered into a checklist, and then, computer for analysis by SPSS version 20 software. Data were analyzed using repeated measurement test at a significance level of 0.05. The present study was approved by the Ethics Committee of Kerman University of Medical Sciences (Ethical code: IR.KMU.1394.335).

Results

In the present study, 10 patients (50%) were under the age of 19 years and 10 patients (50%) were 19 years old and older. Out of these patients, 14 (70%) were female and the rest were male. The mean age of the patients was 19.11 ± 6.23 years. Figure 1 presents the mean and standard deviation of IL-8 level at the beginning of treatment and different time periods. As seen, the level of IL-8 at the baseline was higher than the other time periods. Generally, the level of IL-8 on the first day after treatment significantly decreased compared to the baseline (P = 0.025). There was no statistically significant difference between the age groups below 19 years and 19 years and older in different time periods in terms of IL-8 level. Also, there was no statistically significant difference between gender and time periods in terms of IL-8 level (Table 1). Table 2 presents the mean and standard deviation of IL-8 levels in treatment time periods separately based on the age group of the patients. In the age group of 19 years and older, the level of IL-8 on the first day showed a significant decrease compared to the baseline (P = 0.011). However, no statistically significant difference was observed between the two age groups in terms of IL-8 level, one week and four weeks after treatment.

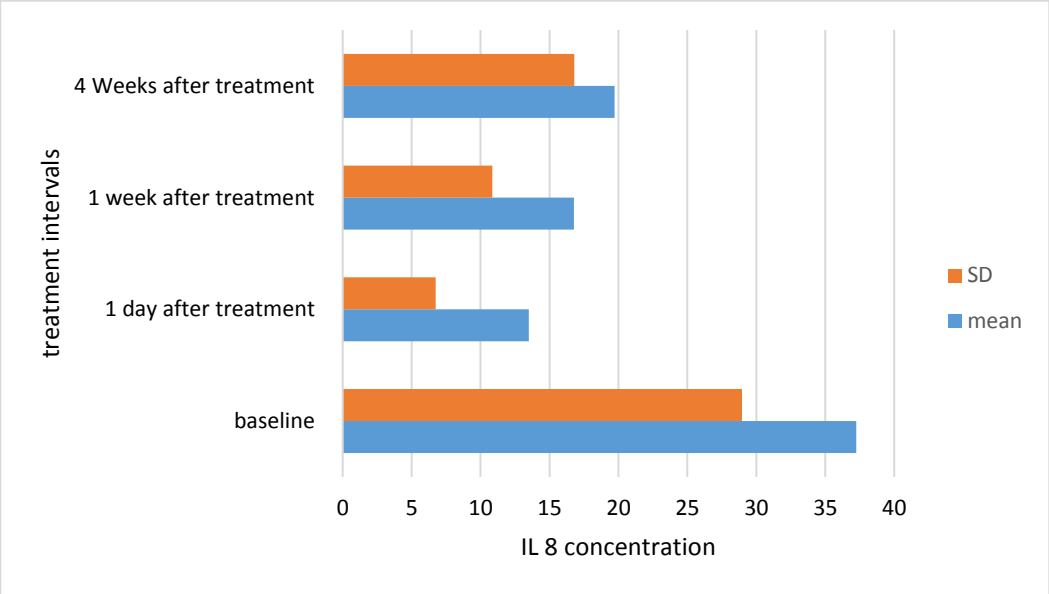

Figure 1. Mean and standard deviation of IL8 concentrations (pg/µL) in different intervals of treatment.

Table 1. Correlation between IL-8 concentration with gender and age group

Gender	Treatment Time				P-value
	Baseline Mean ± SD	1 Day Mean ± SD	1 Week Mean ± SD	4 Weeks Mean ± SD	
			Male		
<19 years old	14.25±14.09	12.61±7.38	10.70±4.60	8.26±5.80	0. 221
≥19 years old	22.00±15.12	14.79±2.73	23.84±6.63	43.44±9.03	0.224
Total	16.72±13.69	13.34±5.96	15.08±8.22	19.98±19.14	0.227
			Female		
<19 years old	49.62±48.60	14.59±9.66	18.71±17.92	14.58±9.17	0.416
≥19 years old	22.64±13.09	12.70±5.18	16.56±4.62	23.78±20.58	0.405
Total	43.51±34.69	13.58±8.43	17.55±12.08	19.58±16.43	0.447

Table 2. Comparison of IL-8 levels in gingival crevicular fluid between adults and adolescents before and after orthodontic treatment

Treatment Time	Age Group (years)	Mean	SD	P-value
Baseline	<19	34.79	30.55	0. 488
	>19	22.50	12.53	
1 Day	<19	13.80	8.437	0.011
	>19	13.16	4.68	
1 Week	<19	15.50	14.23	0.474
	>19	18.18	5.68	
4 Weeks	<19	12.05	8.28	0.594
	>19	28.22	20.06	

Discussion

Mechanical stresses caused by orthodontic appliances stimulate periodontal cells to produce biologically-active substances that are responsible for remodeling connective tissue and activity of osteoclasts. These biological substances can be monitored non-invasively in the GCF (23). In the present study, GCF was used to measure IL-8 during orthodontic movements. Similar studies have shown that GCF is a suitable and non-invasive instrument for measuring mediators (12, 22, 24-26) and has acceptable sensitivity and repeatability in different phases of orthodontic treatment (27). In the present study, the mean IL-8 concentration decreased one day after treatment compared to baseline, and then, increased one week and four weeks after treatment, although it did not reach the baseline level. These results are inconsistent with the results of the study of Kaya et al. (2010), in which the levels of IL-8, IL-2, and IL-6 increased in the time periods after orthodontic treatment compared to the baseline (3). This discrepancy can be attributed to the differences between the time periods studied. In the study of Kaya et al. (2010), the study days were 7 days, 21 days, and 6 months after the beginning of treatment. In the present study, there was no statistically significant difference between IL-8 levels and treatment time periods of 24 hours, one week, and four weeks after treatment, which is consistent with the results of the studies by Chami et al. (2018), which showed no statistically significant difference between IL-1β, IL-7, IL-8, and IL-17 levels and time periods after treatment except the first day (28), and Afshar et al. (22), which showed no significant difference between IL-1β and TNF-α levels and time periods after treatment. Also, Kaya et al. (2010) did not find a statistically significant difference between days 3-7 in terms of TNFα, IL-1β, and IL-8 levels after leveling. It is believed that IL-8 plays a major role in the pathogenesis of various forms of periodontitis, and its high levels is seen in these patients (29). It is a strong pro-inflammatory cytokine that plays a key role in recall and activation of neutrophils in inflammation. They are secreted by monocytes and are involved in regulating bone resorption during orthodontic movement of teeth (30). In the present study, a significant difference was found between the two age groups (adolescents and adults) in terms of the IL-8 concentration on the first day of treatment and baseline. The IL-8 concentration was lower in adults compared to adolescents, although no statistically significant difference was reported between these two age groups totally, which is consistent with the results of other studies, indicating no statistically significant difference between the groups of adults and adolescents in terms of IL-1 and TNF α level (21, 22).

A systematic review and meta-analysis study by Schubert et al. (2020) showed that in most studies, younger people showed faster OTM in the early phase of treatment and the cytokine

level was more significant. Older patients showed a delayed response to orthodontic forces (31). Since the age range of patients in this study was small, the insignificance of IL-8 levels in the two study groups can be justified. In the present study, there was no statistically significant difference between patients' gender and IL-8 concentration during treatment. This result is consistent with the results of other studies that did not show a difference between gender and level of inflammatory cytokines during orthodontic teeth movement (22, 32). In a review article, it was revealed that response to different orthodontic/orthopedic methods does not differ in terms of the patient's gender (33). One of the limitations of this study was small sample size and small age range of the participants, so the results cannot be generalized to all age groups and should be interpreted with caution despite some studies about inflammatory cytokines in OTM, indicating a decrease in the concentration of IL-8 after 30 days (34).

Conclusion

The results of the present study showed that the level of IL-8 significantly decreased on the first day of orthodontic treatment, and then, increased. There was no statistically significant difference between gender and age and IL-8 levels during treatment. Further studies with larger sample sizes and different treatment methods are recommended.

Conflict of Interests

Authors declare that they have no conflict of interests.

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References

1. Li Y, Jacox LA, Little SH H, Ko CC. Orthodontic tooth movement: The biology and clinical implications. Kaohsiung J Med Sci 2018; 34(4):207-14. doi: 10.1016/j.kjms.2018.01.007.
2. Pramustika A, Soedarsono N, Krisnawati, Widayati R. Comparison of tumor necrosis factor-α concentrations in gingival crevicular fluid between self-ligating and preadjusted edgewise appliances in the early leveling stage of orthodontic treatment. Contemp Clin Dent 2018; 9(1):92-6. doi: 10.4103/ccd.ccd_792_17.
3. Kaya FA, Hamamci N, Basaran G, Dogru M, Yildirim TT. TNF-α, IL-1β and IL-8 levels in tooth early levelling movement orthodontic treatment. J Int Dent Med Res 2010; 3:(3):16-121.
4. Baik HS, Kim CK, Lim WH, Chun YS. Interleukin-1α and tumor necrosis factor-α expression on the compressed side of gingiva during orthodontic tooth movement. Open J Stomatol 2012; 2(3):182-7. doi: 10.4236/ojst.2012.23033.
5. Ren Y, Hazemeijer H, de Haan B, Qu N, de Vos P. Cytokine profiles in crevicular fluid during orthodontic tooth movement of short and long durations. J Periodontol 2007; 78:453-8. doi: 10.1902/jop.2007.060261.
6. Meikle MC. The tissue, cellular, and molecular regulation of orthodontic tooth movement: 100 years after Carl Sandstedt. Eur J Orthod 2006; 28(3):221-40. doi: 10.1093/ejo/cjl001.
7. Masella RS, Meister M. Current concepts in the biology of orthodontic tooth movement. Am J Orthod Dentofacial Orthop 2006; 129(4):458-68. doi: 10.1016/j.ajodo.2005.12.013.
8. Jayaprakash PK, Basavanna JM, Grewal H, Modi P, Sapawat P, Bohara PD. 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 Family Med Prim Care 2019; 8(5):1602-6. doi: 10.4103/jfmpc.jfmpc_204_19.
9. Perinetti G, Primozic 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 Craniofac Res 2013; 16(1):1-19. doi: 10.1111/ocr.12005.
10. Capelli J Jr, Fidel Jr R, Figueredo CM, Teles RP. Change in the gingival fluid volume during maxillary canine retraction. Dental Press J Orthod 2010; 15(2):52-7.
11. Yang JH, Li ZC, Kong WD, Zhang W, Jia YP, Zhang YL, et al. Effect of orthodontic force on

inflammatory periodontal tissue remodeling and expression of IL-6 and IL-8 in rats. Asian Pac J Trop Med 2013; 6(10):757-61. doi: 10.1016/S1995-7645(13)60133-9.

12. Giannopoulou C, Mombelli A, Tsinidou K, Vasdekis V, Kamma J. Detection of gingival crevicular fluid cytokines in children and adolescents with and without fixed orthodontic appliances. Acta Odontol Scand 2008; 66(3):169-73. doi: 10.1080/00016350802123126.
13. Gujar AN, Baeshen HA, Alhazmi A, Bhandi S, Raj AT, Patil S, et al. Cytokine levels in gingival crevicular fluid during orthodontic treatment with aligners compared to conventional labial fixed appliances: a 3-week clinical study. Acta Odontol Cand. 2019; 77(6):474-81. doi: 10.1080/00016357.2019.1607548.
14. Dyer GS, Harris EF, Vaden JL. Age effects on orthodontic treatment: adolescents contrasted with adults. Am J Orthod Dentofacial Orthop1991; 100(6):523-30. doi: 10.1016/0889-5406(91)70092-B.
15. Jäger A. Histomorphometric study of age-related changes in remodelling activity of human desmodontal bone. J Anat 1996; 189(Pt 2):257-64.
16. Cei S, Kandler B, Fügl A, Gabriele M, Hollinger JO, Watzek G, et al. Bone marrow stromal cells of young and adult rats respond similarly to platelet-released supernatant and bone morphogenetic protein-6 in vitro. J Periodontol 2006; 77(4):699-706. doi: 10.1902/jop.2006.050155.
17. Norton LA. The effect of aging cellular mechanisms on tooth movement. Dent Clin North Am 1988; 32(3):437-46.
18. Krieger E, Hornikel S, Wehrbein H. Age-related changes of fibroblast density in the human periodontal ligament. Head Face Med 2013; 9:22. doi: 10.1186/1746-160X-9-22.
19. Misawa Y, Kageyama T, Moriyama K, Kurihara S, Yagasaki H, Deguchi T, et al. Effect of age on alveolar bone turnover adjacent to maxillary molar roots in male rats: a histomorphometric study. Arch Oral Biol 2007; 52(1):44-50. doi: 10.1016/j.archoralbio.2006.06.012.
20. Bagga DK. Adult orthodontics versus adolescent orthodontics: an overview. Journal of Oral Health and Community Dentistry 2010; 4(2):42-7. doi: 10.5005/johcd-4-2-42.
21. Rody WJ Jr, Wijegunasinghe M, Wiltshire WA, Dufault B. Differences in the gingival crevicular fluid composition between adults and adolescents undergoing orthodontic treatment. Angle Orthod 2014; 84(1):120-6. doi: 10.2319/012813-85.1.
22. Afshar MK, Safarian F, Torabi M, Farsinejad A, Mohammadzadeh I. Comparison of TNF-α and IL-1β concentrations in gingival crevicular fluid during early alignment stage of orthodontic treatment in adults and adolescents. Pesqui Bras Odontopediatria Clín Integr 2020; 20:e0004. doi: 10.1590/pboci.2020.086.
23. Iwasaki LR, Crouch LD, Tutor A, Gibson S, Hukmani N, Marx DB, et al. Tooth movement and cytokines in gingival crevicular fluid and whole blood in growing and adult subjects. A J Orthod Dentofacial Orthop 2005; 128(4):483-91. doi: 10.1016/j.ajodo.2004.03.037.
24. Ribagin LS, Rashkova MR. Matrix metalloproteinase-8 and interleukin-1beta in gingival fluid of children in the first three months of orthodontic treatment with fixed appliances. Folia Med (Plovdiv) 2012; 54(3):50-6. doi: 10.2478/v10153-011-0097-3.
25. Barbieri G, Solano P, Alarcón JA, Vernal R, Rios-Lugo J, Sanz M, et al. Biochemical markers of bone metabolism in gingival crevicular fluid during early orthodontic tooth movement. Angle Orthod 2013; 83(1):63-9. doi: 10.2319/022812-168.1.
26. Hamamcı 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(3):357-61. doi: 10.1093/ejo/cjr019.
27. Kapoor P, Kharbanda OP, Monga N, Miglani R, Kapila S. Effect of orthodontic forces on cytokine and receptor levels in gingival crevicular fluid: a systematic review. Prog Orthod 2014; 15(1):65. doi: 10.1186/s40510-014-0065-6.
28. Chami VO, Nunes L, Capelli Júnior J. Expression of cytokines in gingival crevicular fluid associated with tooth movement induced by aligners: a pilot study. Dental Press J Orthod 2018; 23(5):41-6. doi: 10.1590/2177-6709.23.5.041-046.oar.
29. Kaya FA, Hamamci N, Basaran G, Dogru M, Yildirim TT. Tnf-A, Il-1β And Il-8 Levels In Tooth Early Levelling Movement Orthodontic Treatment. J Int Dent Med Res 2010; 3: (3), pp. 116-121
30. Tuncer BB, Ozmeriç N, Tuncer C, Teoman I, Cakilci B, Yücel A, et al. Levels of interleukin-8 during tooth movement. Angle Orthod 2005;

75(4):631 doi: 10.1043/0003-3219(2005)75[631:LOIDTM]2.0.CO;2. -6.

31. Schubert A, Jäger F, Maltha JC, Bartzela TN. Age effect on orthodontic tooth movement rate and the composition of gingival crevicular fluid: A literature review. J Orofac Orthop 2020; 81(2):113-25. doi: 10.1007/s00056-019-00206-5.

32. Serra E, Perinetti G, D'Attilio M, Cordella C, Paolantonio M, Festa F, et al. Lactate dehydrogenase activity in gingival crevicular fluid during orthodontic treatment. Am J Orthod Dentofacial Orthop 2003; 124(2):206-11. doi: 10.1016/s0889-5406(03)00407-4.

33. Lagorsse A, Gebeile-Chauty S. Does gender make a difference in orthodontics? A literature review. Orthod Fr 2018; 89(2):157-68. doi: 10.1051/orthodfr/2018011. [In French].

34. Tuncer BB, Ozmeriç N, Tuncer C, Teoman I, Cakilci B, Yücel A, et al. Levels of interleukin-8 during tooth movement. Angle Orthod 2005; 75(4):631 doi: 10.1043/0003-3219(2005)75[631:LOIDTM]2.0.CO;2. -6.