International Journal Dental and Medical Sciences Research (IJDMSR)
ISSN: 2393-073X Volume 4, Issue 3 (March-2020), PP 24-30
www.ijdmsr.com

Evaluation of Periodontal Health Changes and measurement of Interleukin-1 and Interleukin-6 in Gingival Crevicular Fluid after Orthodontic Band and Bond during Orthodontic Treatment

Talab A.A. Alqarari[1], Jing Mao[1],
[1]*Department of Stomatology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, China.*
**Corresponding Author: Prof/ Jing Mao*

ABSTRACT:-
Objective: The aim of this study was to investigate the periodontal status of orthodontic bands relative to orthodontic bonds.
Methods: The sample consisted of 28 patients aged 13–17 years who were set to receive fixed orthodontic treatment in both arches at an orthodontic clinic. A total of 84 molars were set for treatment. The following inclusion criteria were considered: no history of rheumatic fever, congenital heart disease, blood dyscrasias, diabetes mellitus, or juvenile periodontitis; no antibiotic therapy or use of oral irrigators or topical chemical agents; and no inhibition of plaque during the previous 6 months. Patients requiring arch expansion or molar distalization with auxiliary appliances were excluded because these additional appliances might interfere with oral hygiene practices. The participants expressed willingness to undergo periodontal treatment prior to orthodontic treatment if necessary. A total of 56 first molars from the mandibular comprised the experimental group, and 28 molars made up the control group. The experimental group was divided into the molar band groups and buccal tube groups, in addition to a control group without a fixed appliance.
IL-1 and IL-6 were tested by ELISA test .
Results: Interleukin-1 and interleukin-6 increased significantly after 6 and 12 months of orthodontic treatment in the band and bond groups relative to the control group ($p < 0.01$). Interleukin-1 and interleukin-6 also varied significantly between the two experimental groups ($p < 0.01$).
Conclusion: Periodontal health is an important consideration in fixed orthodontic treatment. The careful maintenance of oral hygiene and periodic periodontal consultations are essential for orthodontic patients. Clinical results suggest that the placement of molar bands could be more damaging to periodontal health than the use of buccal tubes.

Key Words: Interleukin-1, Interleukin-6, Orthodontic bands, Orthodontics bonds, Gingival crevicular fluid

I. INTRODUCTION

The association between orthodontic bands and periodical diseases has been handled by many researchers. Some concluded that these bands may cause gingival inflammation soon after being fixed [1-5] especially in interproximal sites and in posterior teeth particularly rather than anterior [6]. Some attributed that to specific reasons including the mechanical irritation of the bands, chemical irritation from cement material, the risk of food impaction and at last but not the least the tenancy of patients in cleaning their interior teeth effectively more than the posterior.

In an assessment study on banded an bonded teeth based on gingival inflammation, loss of attachment and plaque accumulation, the authors indicated no significant difference in gingival inflammation during the pre-treatment stage [7], while both maxillary and mandibular banded teeth were significantly affected by gingival inflammation and plaque accumulation along with loss of attachment against bonded molar later with the continuity of the treatment.

Following the changes in subgingival microflora in children after band placement [8], the findings highlighted that the percentage of black-pigmented Bacteroides was increased among children under study. A result from the clinical trial between two groups also revealed an increase in fusiform, motile rods spirochetes and filament percentages in the group with bands rather than the other with no bands [9].

A three years study in which a particular culture method was involved exhibited significant rates in harbor Actinobacillusactinomycetemcomitansto among young patients than in matched control [10].

The advantages of using bonds over bands include unnecessary various sized bands, no requirements for separated appointments and nonoccurrence of extensive pain accompany the separation visit [11], accordingly, the prevention or reduction of the effects of bands is essential in medically compromised individuals particularly

The orthodontic tooth movement in its early stage includes a severe provocative response at both biochemical and structural levels featured by the migration of leukocytes out of periodontal ligament capillaries and periodontal vasodilatation [13]. The orthodontic tooth movement is considered an epiphenomenon of the gene expression of the periodontal ligament surrounding cells that emerge due to orchestrated cellular and molecular occurrences in alveolar bone and periodontal launched with orthodontic force application [14]. The bone resorption mechanism may also be associated with the release of inflammatory mediators such as prostaglandin E and interleukin-1, which interact with bone cells.

Leukocytes discharge cytokines that may directly or indirectly interact with osteoblasts or neighboring cells such as monocytes/ macrophages, lymphocytes, and fibroblasts respectively. A chemical substance that facilitates signal conveyance from the extracellular matrix is arbitrated by mediators releasing in the paracrine environment, these signals change the cytoskeletal structure within turn modifies the nuclear protein matrix and gene suppression initiating bone remodeling process and resulting in effective tooth movement [15, 16]. The biochemical mediators during orchestration of tooth movements exist in gingival crevicular fluid (GCF), which is a distinct biological medium for studying mediators within an acceptable sensitivity. GCF can be obtained either noninvasively or frequently during orthodontic treatment [5].

The success of orthodontic tooth movement is related to expression molecules of which cytokines are documented. Cytokines showed available in autocrine environments reacted by local signals as stress [17] they are also entangled with normal physiological bone turnover and remodeling [18-20]. Biologically, the cytokine is hard to comprehend because of the redundancy and pleiotropy caused by sheer number and intricacy of these factors [21]. Due to their contribution to bone and tissue remodeling, there is an ongoing concern to comprehend the role of cytokines as mediators of orthodontic tooth movement in spite of being evaluated as quantitative biochemical indicators of inflammatory
periodontal status [22].

IL-1a, IL-1ß, IL-1RA, IL-2, IL-6, and IL-8, tumor necrosis factors, interferons, growth factors, and colony-stimulating factors cytokines and their relation to orthodontic tooth movement have been extensively investigated in this study aiming to identify and quantify the Interleukin -1 and Interleukin -6 in gingival crevicular fluid (GCF), and to investigate the changes in their levels during orthodontic band and buccal tune.

II. MATERIALS and METHODS

III. 3.1 Material

3.1.1 Orthodontic band and orthodontic buccal tube
3.1.2 Paper points Absorbentno.30-Eppendorf Low temperature(5804R**)**
3.1.3 Fuchsin basic sodium tetraborate
3.1.4 12 pipettes Eppendorf AG

3.2 Methods:

3.2.1 Data collection:

The sample consisted of 28 patients, 84 molars, was a collection in the orthodontics department, from May 2016 to June 2018, to investigate the periodontal status of orthodontic bands relative to orthodontic bonds. aged 13–17 years.

The patients were selected according to the following criteria: no history of rheumatic fever, congenital heart disease, blood dyscrasias, diabetes mellitus or juvenile periodontitis .no antibiotic therapy or use of oral irrigators or topical chemical agents know no inhibit plaque during the previous 6 months3). The selected patients then received fixed orthodontic treatment in both arches at the Orthodontic Clinic. Patients requiring arch expansion or distalization of molars with auxiliary appliances were excluded due to the possibility of intervention of these additional appliances with oral hygiene.

Dividing the studded sample into two groups, 56 first molar was selected from mandibular as an experimental group against 28 molars from maxillary as a control group. The experimental group also divided into two groups including the molar band group and the buccal tube group compared to a control group without a fixed appliance.

3.2.2 Microbial sampling GCF

Cotton tissues were used to gently isolate the teeth from salvia and dry them to avoid contamination, then after, a sterile curette was implemented to remove the supragingival plaque carefully without traumatizing the gingiva to decrease the production of gingival crevicular fluid (GCF); [23].
The supragingival plaque was then moved into flip-capped vials containing 2.0 ml pre-reduced transport fluid

[24]. Each sample was homogenized by vertexing for 30 seconds and coded. After the analysis was terminated, the coding was revealed leading to blinded microbiological analyses. After collecting the GCF, the subgingival plaque was sampled to avoid the crevice traumatizing.
Six sterile medium paper points (RoekoA, Roeko, Langenau, Germany) were inserted per site (three mesially and three distally) and kept in place for at least 10 seconds. The subgingival plaque samples were processed similarly to the supragingival samples [25].

3.2.3 Measurement of interleukin (IL)-1 and IL-6

Enzyme-linked immunosorbent test (ELISA) was used for measuring Interleukin-1 and interleukin-6. all reagents and samples were in room temperature (≈25°) before use. Before the assay, the sample was centrifuged again after defrost.

It is advised to double analyzing all samples and standards by preparing all reagents, working standards and samples assigned in the previous section, then determining the number of Wells to be used and restore the rest of the Wells and desiccant to the bag, seal Ziploc, unused Wells were to be stored at 4 degrees Celsius, adding 100% C at 4C C and 100% standard sample and get covered with the adhesive belt provided. For recording standards and samples, a plate layout that incubates for 37 hours at 37C Celsius was prepared. the liquid in each bore was then removed.

100 mg biotin antibody-1x was added to each bore. the new adhesive strip was covered and incubated at 37 C for 1- hour (biotin antibody 1x may be cloudy). The room was heated up to temperature until stirred. Bottles were washed in 200 L by injecting bottles with a spouted bottle, multi-channel pipette, manifold di Spenser, or automatic washing machine, and for 2 minutes to remove the liquid completely in each step for good performance Indispensable. After the last cleaning, the remaining cleaning buffer was removed by suction or germination.The plate was flipped and blocked against a clean paper towel. 100 y of HRP avidin-1x were added to each well. the microtiter plate was covered with a new sticky plate. After incubating the samples for 1 hour at 37 ° C, the suction and cleaning treatment was repeated 5 -times as in step 6. then after the incubation of 90 l of TMB substrate at 37 degrees for 15 to 30 minutes, it was added to each bore. then 50- solution l stop solution was added to each well and the plate was gently tapped to ensure full mixing.

We used a microplate reader set to 450 nm to identify the optical density of each well in 5-minutes time, when wavelength correction is possible, it was set to 540 nm or 570 nm. The value of measurement was subtracted from the measurement of 450nm at 540 nm or 570nm after the process. This subtraction was then corrected for optical defects in the plate.

3.2.5 Statistics

Paired t-test was used in comparison of IL-1, IL-6 levels of pre/post-treatment in each patient, and t-test was used in comparison between two groups. ANOVA test was used for comparison of more than 2 groups

CHAPTER III

IV. RESULTS

Table 3.1 Determine interleukin-1 During the Study Period (Mean ± S.D)

	Before treatment	6 months	12 months
Bands group	0.319±0.011	1.002±0.014	1.380±0.015
Bonds group	0.316±0.010	0.705±0.010	0.813±0.013
Control group	0.314±0.010	0.315±0.012	0.318±0.018

Table: 3.1 shows that the content of interleukin-1 did not change in the gingival crevicular fluid and that the mean values of the three groups of cases did not present any differences. After 6 months of orthodontic treatment, their oral health worsened. Interleukin-1 increased significantly relative to the baseline ($p < 0.01$). A significant difference was noted between the molar band group and the buccal tube group ($p < 0.01$).
After 12 months of treatment, the gum condition worsened, and interleukin-1 continued to rise relative to the baseline. Both experimental groups presented significant differences ($p < 0.01$). A significant difference was noted between the molar band group and the buccal tube group ($p < 0.01$).

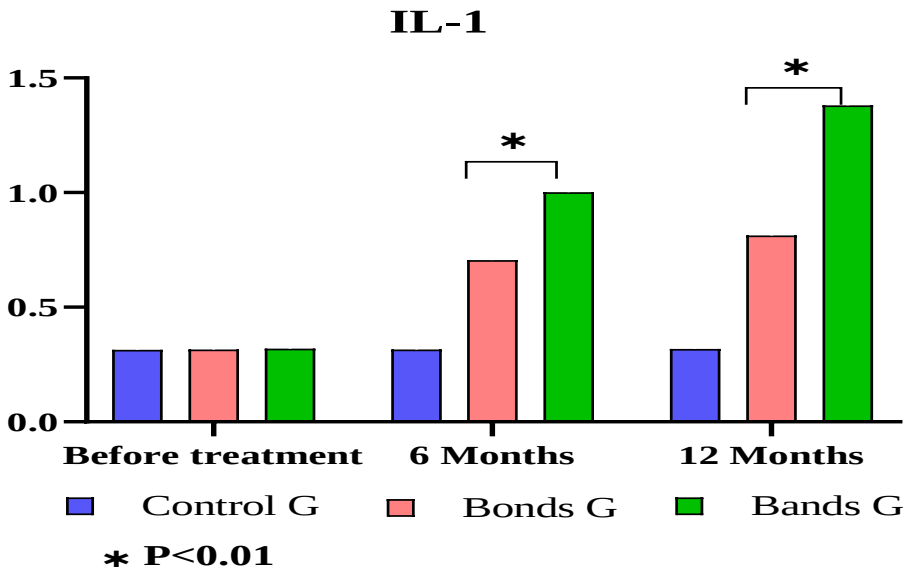

Figure 1: Interleukin-1 Before Treatment and After 6 And 12 Months in Three Groups

Table 3.2 Determine interleukin-6 During the Study Period (Mean ± S.D)

	Before treatment	6 months	12 months
Bands group	0.213±0.017	0.936 ±0.009	1.271± 0.011
Bonds group	0.215 ±0.020	0.569 ±0.012	0.609± 0.021
Control group	0.212 ±0.010	0.215 ±0.011	0.217 ±0.019

Table 3.2 shows that the content of interleukin-1 did not change in the gingival crevicular fluid and that the mean values of the three groups of cases did not present any differences. After 6 months of orthodontic treatment, their oral health worsened, and interleukin-1 increased significantly relative to the baseline ($p < 0.01$). A significant difference was noted between the molar band group and the buccal tube group ($p < 0.01$).

After 12 months of treatment, the gum condition worsened, and interleukin-6 continued to rise relative to the baseline. Both experimental groups presented significant differences ($p < 0.01$). A significant difference was noted between the molar band group and the buccal tube group ($p < 0.01$)

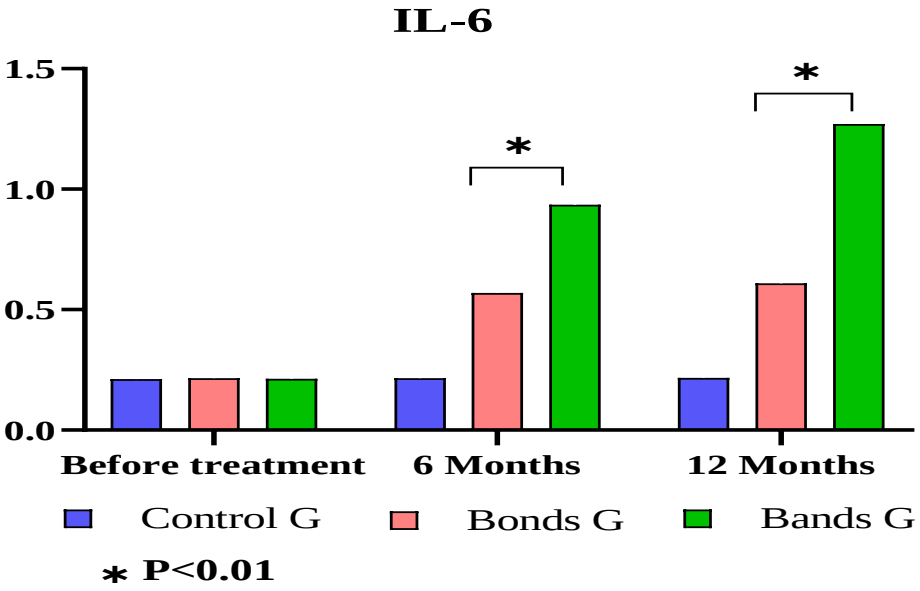

Figure 2: Interleukin-6 Before Treatment and After 6 And 12 Months in Three Groups

V. DISCUSSIONS

On the other hand, the response to inflammation is observed by determining inflammatory cytokines, which indicates that the levels of secondary IL-1 and IL-6 have become more during the treatment. The molar bands IL-1 and IL-6 are also higher than buccal tubes.

These results show that the two methods (molar band and buccal tube) may have influence on the health of Orthodontic tooth, However, when using buccal tubes, the influence decreased. Our study was in accordance with the previous studies. For example, Robert *et. al*[7] reported an obvious plaque accumulation and gingival inflammation at inter-proximal sites of banded molars than interproximal sites of bonded molars during orthodontic treatment of adults and adolescents. Orthodontic bands cause periodontal inflammation.

Theoretically, buccal tube (bond) involvement supposed to decrease periodontal changes more than a band because they placed out of the gingival margins. The results of this research have shown the trend to support the hypotheses and findings of previous similar papers in general; the greatest loss of attachment were found in the banded group, invariably to the subjects with the poorest plaque removal who had a high levels of gingival inflammation.

This observation is in conformity with those of previous studies[26-28]. Measurements of the inflammatory cytokines IL-1 and IL-6 increased during treatment. The increments in IL-1 and IL-6 in bands were also higher than those in bonds. Gingival crevicular fluid (GCF) was introduced for the first time by periodontists to prepare a diagnostic test for periodontal diseases. The watery texture of GCF is primarily derived from serum, gingival tissues through which the fluid passes, and bacteria was found in the tissues and gingival sulcus.

In this study, the selection of GCF was due to its accessibility and harmlessness. The IL-1 and IL-6 levels, as detected by ELISA, were measured as total cytokine mass per GCF volume secreted in 60 s per strip and are expressed in picogram per 60 seconds.

Similar to other authors, our research group believes that in the described manner the number of cytokines in GCF, secreted by periodontal tissues, is presented most realistically [29, 30], and taken most accurately, considering the specific need for the concentration to be expressed in picogram per liter GCF or picogram per gram protein of GCF [31] This study showed that the buccal tube and molar band affected periodontal health.

Our findings also agreed with those of Amila and Aleksandra [32] who found that GCF IL-1 and IL-6 expression levels increase during the initial phase of OTM in children and adults. However, the levels of these cytokines, especially IL-6, increase more rapidly in juveniles than in young adults. Thus, the initial OTM is faster in juveniles than in adults.

In patients with severe root resorption, Ryuichi *et al*.. [33] detected a high level of IL-6 in the GCF. They also found that heavy CF induces IL-6 production and stimulates osteo/odontoclastogenesis after orthodontic treatment, suggesting that IL-6 may be a biomarker for root resorption and that IL-6 may facilitate OIIRR[34], Meanwhile, the current study suggested that IL-1Ra down-regulated OTM probably through its anti-inflammatory activity.

Moreover, previous studies [35-37]. have shown that the level of IL-1ß significantly exceeds that of IL-6 in the fluid of treated teeth. Our results indicated that IL-1 and IL-6 levels in GCF during treatment showed a bimodal peak in molar band and buccal-tube groups. However, this increase became more prominent in the molar-band group after 6 months and in the buccal tube after 12 months.

The IL-1 level was significantly higher at the very beginning of OTM and 12 months into treatment in GCF samples of the buccal-tube group compared with those of the molar-band group. These findings were consistent with previously reported data on increased levels of proinflammatory cytokines, including IL-1 and IL-6, in GCF during human OTM [38, 39].

The current results also showed the presence of IL-1ß, IL-6, and TNF-a in the GCF of late-adult rats. The cytokine levels in teeth of late-adult rats applied with orthodontic force were similar to those in young rats [35]. Based on these results, we believe that CGRP and SP may be involved in pulp inflammation that occurs during dental correction.

VI. CONCLUSIONS

From this study, we can conclude that strict oral hygiene instructions and frequent oral prophylaxis by professionals can reduce gingivitis and gingival enlargement in patients who are under fixed orthodontic treatment.

Periodontal health is an important consideration in fixed orthodontic treatment. Careful maintenance of oral hygiene and periodic periodontal consultation are essential for orthodontic patients. The clinical results suggest that the placement of molar bands could be more damaging to periodontal health than the buccal tube.

We recommend the use of buccal tube in orthodontics because it is better for periodontal health

REFERENCES

[1]. Baer, P.N. and P.J. Coccaro, Case Report….: Gingival Enlargement Coincident with Orthodontic Therapy. Report of Three Cases. The Journal of Periodontology, 1964. **35**(5): p. 436-439.

[2]. ZAGHRISSON, B.U. and S. ZACHRISSON, Caries incidence and oral hygiene during orthodontic treatment. European Journal of Oral Sciences, 1971. **79**(4): p. 394-401.

[3]. KLOEHN, J.S. and J.S. PFEIFER, The effect of orthodontic treatment on the periodontium. The Angle Orthodontist, 1974. **44**(2): p. 127-134.

[4]. Sanders, N.L., Evidence-based care in orthodontics and periodontics: a review of the literature. The Journal of the American Dental Association, 1999. **130**(4): p. 521-527.

[5]. Naranjo, A.A., et al., Changes in the subgingival microbiota and periodontal parameters before and 3 months after bracket placement. American Journal of Orthodontics and Dentofacial Orthopedics, 2006. **130**(3): p. 275. e17-275. e22.

[6]. Zachrisson, B.U., Cause and prevention of injuries to teeth and supporting structures during orthodontic treatment. American Journal of Orthodontics and Dentofacial Orthopedics, 1976. **69**(3): p. 285-300.

[7]. Boyd, R.L. and S. Baumrind, Periodontal considerations in the use of bonds or bands on molars in adolescents and adults. The Angle Orthodontist, 1992. **62**(2): p. 117-126.

[8]. Diamanti‐Kipioti, A., F.A. Gusberti, and N.P. Lang, Clinical and microbiological effects of fixed orthodontic appliances. Journal of Clinical Periodontology, 1987. **14**(6): p. 326-333.

[9]. Huser, M.C., P.C. Baehni, and R. Lang, Effects of orthodontic bands on microbiologic and clinical parameters. American Journal of Orthodontics and Dentofacial Orthopedics, 1990. **97**(3): p. 213-218.

[10]. Paolantonio, M., et al., Clinical significance of Actinobacillus actinomycetemcomitans in young individuals during orthodontic treatment: A 3‐year longitudinal study. Journal of clinical periodontology, 1997. **24**(9): p. 610-617.

[11]. Ngan, P., et al., The effect of ibuprofen on the level of discomfort inpatients undergoing orthodontic treatment. American Journal of Orthodontics and Dentofacial Orthopedics, 1994. **106**(1): p. 88-95.

[12]. Burden, D., B. Mullally, and J. Sandler, Orthodontic treatment of patients with medical disorders. The European Journal of Orthodontics, 2001. **23**(4): p. 363-372.

[13]. Davidovitch, Z., et al., Neurotransmitters, cytokines, and the control of alveolar bone remodeling in orthodontics. Dental Clinics of North America, 1988. **32**(3): p. 411-435.

[14]. Gianni, E., Genetics and dynamical modulators in orthodontics. WFO Gazzette, 2013. **18**(2): p. 4.

[15]. Meikle, M.C., The tissue, cellular, and molecular regulation of orthodontic tooth movement: 100 years after Carl Sandstedt. The European Journal of Orthodontics, 2006. **28**(3): p. 221-240.

[16]. Masella, R.S. and M. Meister, Current concepts in the biology of orthodontic tooth movement. American Journal of Orthodontics and Dentofacial Orthopedics, 2006. **129**(4): p. 458-468.

[17]. Meager, A., Cytokine regulation of cellular adhesion molecule expression in inflammation. Cytokine & growth factor reviews, 1999. **10**(1): p. 27-39.

[18]. Saito, S., et al., Effects of parathyroid hormone and cytokines on prostaglandin E synthesis and bone resorption by human periodontal ligament fibroblasts. Archives of oral biology, 1990. **35**(10): p. 845-855.

[19]. Mundy, G.R., Cytokines and local factors which affect osteoclast function. The International Journal of Cell Cloning, 1992. **10**(4): p. 215-222.

[20]. Kimoto, S., et al., Cytokine secretion of periodontal ligament fibroblasts derived from human deciduous teeth: effect of mechanical stress on the secretion of transforming growth factor‐β1 and macrophage colony stimulating factor. Journal of periodontal research, 1999. **34**(5): p. 235-243.

[21]. Ozaki, K. and W.J. Leonard, Cytokine and cytokine receptor pleiotropy and redundancy. Journal of Biological Chemistry, 2002. **277**(33): p. 29355-29358.

[22]. Yue, Y., et al., Comparative evaluation of cytokines in gingival crevicular fluid and saliva of patients with aggressive periodontitis. The International journal of biological markers, 2013. **28**(1): p. 108-112.

[23]. Nonaka, S., et al., Randomization of left–right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. Cell, 1998. **95**(6): p. 829-837.

[24]. Syed, S.A. and W.J. Loesche, Survival of human dental plaque flora in various transport media. Appl. Environ. Microbiol., 1972. **24**(4): p. 638-644.

[25]. Van Gastel, J., et al., Influence of bracket design on microbial and periodontal parameters in vivo. Journal of clinical periodontology, 2007. **34**(5): p. 423-431.

[26]. ZACHRISSON, B.U. and L. ALNAES, Periodontal condition in orthodontically treated and untreated individuals I. Loss of attachment, gingival pocket depth and clinical crown height. The Angle Orthodontist, 1973. **43**(4): p. 402-411.

[27]. Sjølien, T. and B.U. Zachrisson, Periodontal bone support and tooth length in orthodontically treated and untreated persons. American Journal of Orthodontics, 1973. **64**(1): p. 28-37.
[28]. Hamp, S.-E., F. Lundström, and S. Nyman, Periodontal conditions in adolescents subjected to multiband orthodontic treatment with controlled oral hygiene. The European Journal of Orthodontics, 1982. **4**(2): p. 77-86.
[29]. Boyd, R., et al., Periodontal implications of orthodontic treatment in adults with reduced or normal periodontal tissues versus those of adolescents. American Journal of Orthodontics and Dentofacial Orthopedics, 1989. **96**(3): p. 191-198.
[30]. Giannopoulou, C., et al., Detection of gingival crevicular fluid cytokines in children and adolescents with and without fixed orthodontic appliances. Acta Odontologica Scandinavica, 2008. **66**(3): p. 169-173.
[31]. Dudic, A., et al., Composition changes in gingival crevicular fluid during orthodontic tooth movement: comparisons between tension and compression sides. European journal of oral sciences, 2006. **114**(5): p. 416-422.
[32]. Filiz, A., et al., The gingival crevicular fluid levels of IL-1β, IL-6 and TNF-α in late adult rats. International Dental Research, 2011. **1**(1): p. 7-12.
[33]. Vujačić, A., et al., Differences in IL-1β and IL-6 levels in the gingival crevicular fluid during acute phase of orthodontic tooth movement between juveniles and young adults. Vojnosanitetski pregled, 2017. **74**(3): p. 219-226.
[34]. Kunii, R., et al., Role of interleukin-6 in orthodontically induced inflammatory root resorption in humans. The Korean Journal of Orthodontics, 2013. **43**(6): p. 294-301.
[35]. Salla, J.T., et al., The effect of IL-1 receptor antagonist on orthodontic tooth movement in mice. Archives of oral biology, 2012. **57**(5): p. 519-524.
[36]. Uematsu, S., M. Mogi, and T. Deguchi, Interleukin (IL)-1β, IL-6, tumor necrosis factor-α, epidermal growth factor, and β2-microglobulin levels are elevated in gingival crevicular fluid during human orthodontic tooth movement. Journal of dental research, 1996. **75**(1): p. 562-567.
[37]. Ren, Y., et al., Cytokine profiles in crevicular fluid during orthodontic tooth movement of short and long durations. Journal of periodontology, 2007. **78**(3): p. 453-458.
[38]. Grant, M., et al., Induction of cytokines, MMP9, TIMPs, RANKL and OPG during orthodontic tooth movement. European journal of orthodontics, 2012. **35**(5): p. 644-651.
[39]. Zhang, D. and Y. Ren, Comparison of GCF biochemical components changes during orthodontic tooth movement between children and adults. Zhonghua kou qiang yi xue za zhi= Zhonghua kouqiang yixue zazhi= Chinese journal of stomatology, 2001. **36**(3): p. 219-221.

Corresponding Author: Prof/ Jing Mao
[1]Department of Stomatology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, China.