Effect of orthodontic tooth movement on salivary levels of Interleukin-1beta, Tumor Necrosis Factor-alpha, and C-reactive protein

Noor Saadi, B.D.S. [(1)]
Nidhal H. Ghaib, B.D.S., M.Sc. [(2)]

ABSTRACT

Background: Orthodontic force is considered to stimulate cells in the periodontium to release many mediators such as cytokines which play a responsible role for periodontal and alveolar bone remodeling, bone resorption and new bone deposition. Aim of this study was carried out to estimate changes of the (interleukin-one beta, tumor necrosis factor – alpha and C-reactive protein) levels in unstimulated whole saliva during the leveling stage of orthodontic tooth movement.

Materials and methods: The sample consisted of thirty adult patients (12 males and 18 females) with ages ranges (19-23) years. Each sample had Class I and Class II malocclusion dental classification and required bilateral extraction of their maxillary first premolars, underwent a session of professional oral hygiene and received oral hygiene instructions before and during the period of study, one month later fixed orthodontic appliance were placed in the upper arch by using 0.014 nickel titanium arch wire. The unstimulated whole saliva was taken from each sample immediately before placement of the appliance (baseline), and at (after1hour, after one week and after two week) following placement of the fixed orthodontic appliance. In addition the plaque index and gingival index were recorded during the interval periods of this study to assess oral cleanliness. The interleukin – one beta and tumor necrosis factor-alpha were determined by enzyme linked immunosorbent assay, while the C-reactive protein was determined by latex agglutination.

Results: The results of the present study found the mean value of both salivary (interleukin-one beta and tumor necrosis factor -alpha) were highest at (after1hour) followed by at (after one week) then at (after two week) than the baseline with highly statistical significant differences (P< 0.01) among males, females and total samples, in addition there were no statistical significant differences between males and females (P>0.05). While the mean values of C-reactive protein were higher at (after 1hour) only with highly significant differences (P< 0.01) among females and total samples while only significant difference (P<0.05) for males, in addition there were no statistical significant differences between males and females. Regarding the correlation between salivary (interleukin – one beta and tumor necrosis factor -alpha), there were positive correlation between them at all periods of study. Moreover there were positive correlation between salivary (interleukin – one beta and tumor necrosis factor -alpha) and salivary C-reactive protein. On the other hand there were no association between the salivary (interleukin – one beta and tumor necrosis factor -alpha and C-reactive protein) and clinical parameter (plaque index and gingival index).

Conclusion: From this clinical study we conclude that orthodontic force induces increasing the levels of (interleukin – one beta, tumor necrosis factor -alpha, C-reactive protein) in unstimulated whole saliva during orthodontic tooth movement.

Keywords: interleukin-one beta, tumor necrosis factor – alpha, C-reactive protein, unstimulated saliva, orthodontic tooth movement. (J Bagh Coll Dentistry 2013; 25(4):120-125).

INTRODUCTION

Orthodontic tooth movement is based on force that stimulate periodontal ligaments and alveolar bone remodeling, at the early stage of orthodontic tooth movement, the host response to orthodontic force has been described as an aseptically and transitory inflammation characterized by alteration the vascularity and blood flow of periodontal ligament (PDL), resulting in local synthesis and release of different mediators such as cytokines (IL-1β, TNF-α, etc.) involved in alveolar bone remodeling process [(1-4)].Importantly, IL-1β is pro-inflammatory cytokines produced by the periodontal ligament (PDL) in sufficient quantities to diffuse into the gingival crevicular fluid (GCF) and has been identified as a biomarker of orthodontic tooth movement [(5-8)].

TNF-α is a typical mediator of inflammatory response that has been shown to be involved in the process of bone resorption and to be locally elevated in response to orthodontic force. It plays a prominent role in the mechanism controlling the appearance of osteoclasts at compression sites [(8-14)]. Furthermore cytokines such as (IL-1β and TNF-α) produced at the gingival sites may be transported into the systemic circulation and stimulate hepatocytes in the liver to produce C – reactive protein (CRP), which provide a biomarker for low grad systemic inflammation [(15-19)].Inflammatory cytokines such as IL-1β and TNF-α which are involved in bone and periodontal remodeling, have been quantified in the salivary crevicular fluid of patients undergoing orthodontic treatment [(7-8,11,20)]. Different mediators involved in alveolar bone remodeling are continuously washed into saliva by GCF, whole-saliva samples may constitute an easy alternative to individual gingival sulcular

(1)Master student. Department of Orthodontics. College of Dentistry. University of Baghdad
(2)Professor. Department of Orthodontics. College of Dentistry. University of Baghdad

samples for determining analytes of bone turnover that are present within the periodontal environment, providing a sensitive and inexpensive detection technique [21].

Aim of this study was carried out to estimate changes of the (interleukin-one beta, tumor necrosis factor – alpha and C-reactive protein) levels in unstimulated whole saliva during the leveling stage of orthodontic tooth movement.

MATERIAL AND METHODS

Subjects recruited for the study were volunteer patients attending the orthodontic department at the College of Dentistry University of Baghdad seeking for orthodontic treatment. A total of 30 adult patients (12 males and 18 females) with an age range of (19-23 years) were participated in the study. They should be with Class I and /or Class II malocclusion cases requiring bilateral extraction of their maxillary first premolar teeth. Each subject prior the placement of the orthodontic appliance should extracted both upper first premolars (right and left) at least 20 days**.** During this period, all the subjects should be with good oral hygiene[22]**.** The collection of the unstimulated salivary samples from the individuals was formed under standardized conditions[23-24]. In the current study saliva collected between 9-12 am at different times; before placement of the orthodontic appliance(1hour) as a baseline (T0), then 1 hour after placement of orthodontic appliance (T1), one week (T2), two week (T3). The samples were stored at -20^{o}c in a deep freeze until processed [25]**.**

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for IL-1β has been pre-coated onto a microplate. Standards and samples were pipetted in to the wells and any IL-1β present was bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for IL-1β was added to the wells. After washing, Avidin conjugated Horseradish peroxidase (HRP) was added to the wells. Following a washed to remove any unbounded Avidin-enzyme reagent, a substrate solution was added to the wells and color develops in proportion to the amount of IL-1β bound in the initial step. The color development was stopped and the intensity of the color was measured. C-reactive protein in saliva was detected by using latex agglutination slide test (Human Tex CRP) for the qualitative and semi-quantitative determination of C-reactive protein in non - diluted saliva.

Principle: Human tex CRP was based on the immunological reaction between human C-reactive protein (CRP) of patient specimen or control serum and the corresponding anti- human CRP antibodies bound to latex particles. The positive reaction is indicated by distinctly visible agglutination of the latex particles in the test cell of the slide.All data analyses were performed using the SPSS statistical software program (version 10 for Windows, SPSS). The confidence level was accepted at the level of 5%.

RESULTS

Results showed that the mean value of salivary IL-1β (pg/ml) and TNF-α (pg/ml) were highest at T1, followed by T2, then T3 and then T0 with highly statistically significant difference (P< 0.01) among males, females, and total sample as shown in **(table 1)** and **(table 2)** respectively .According the T-test, the mean value of salivary IL-1β (pg/ml) among males was higher than females with no statistically significant difference (P > 0.05) at T0, T1, T2, and T3. The same results was recorded concerning TNF-α (pg/ml).

Table 1. Mean of IL-1β concentration (pg/ml) among samples

		IL-1β pg/ml															
		Baseline (T0)				After 1 hour (T1)				After 1 week (T2)				After 2 week (T3)				ANOVA Test	
		Mean	SD	T-test		Mean	SD	T-test		Mean	SD	T-test		Mean	SD	T-test			
Gender	No.			t	Sig.			t	Sig.			t	Sig.			t	Sig.	F	Sig.
Males	12	161.99	6.19			435.02	22.39			260.64	11.02			234.35	10.02			845.031	0.000 **
Females	18	156.82	7.31	2.014	0.054	422.97	26.79	1.286	0.209	251.37	12.00	2.041	0.051	226.33	11.08	2.016	0.053	867.412	0.000 **
Total	30	158.89	7.25			427.79	25.44			255.08	12.84			229.54	11.23			1577.978	0.000 **

No significant= P value > 0.05. *(Significant)= P value < 0.05.(Highly significant)= P value < 0.01.**

Table 2. Mean of TNF-α concentration (pg/ml) among samples:

		TNF-α pg/ml															
		Baseline (T0)				After 1 hour (T1)				After 1 week (T2)				After 2 week (T3)				ANOVA Test	
		Mean	SD	T-test		Mean	SD	T-test		Mean	SD	T-test		Mean	SD	T-test			
Gender	No.			t	Sig.			t	Sig.			t	Sig.			t	Sig.	F	Sig.
Males	12	2.07	0.03			5.98	0.17			3.69	0.06			2.95	0.05			3867.301	0.000 **
Females	18	2.00	0.11	1.877	0.071	5.67	0.57	1.798	0.083	3.57	0.21	1.810	0.081	2.86	0.16	1.858	0.074	430.568	0.000 **
Total	30	2.03	0.10			5.80	0.48			3.62	0.17			2.90	0.14			1096.92	0.000 **

Non significant= P value > 0.05. *(Significant)= P value < 0.05. **(Highly significant)= P value < 0.01.

As demonstrated in (Table 3)the mean value of salivary CRP (mg/l) was highly at the T1 with highly statistically significant difference (P< 0.01) among females and total sample and only significant difference (P<0.05) for males. The present study reported the normal of salivary CRP at the period (T0, T2, and T3) for both males and females. According to T-test, the mean value of salivary TNF-α (pg/ml) among females was higher than males with no statistically significant difference (P > 0.05) at T1.

Table 3. Mean of CRP concentration (mg/l) among samples

		CRP mg/l															
		Baseline (T0)				After 1 hour (T1)				After 1 week (T2)				After 2 week (T3)				ANOVA Test	
		Mean	SD	T-test		Mean	SD	T-test		Mean	SD	T-test		Mean	SD	T-test			
Gender	No.			t	Sig.			t	Sig.			t	Sig.			t	Sig.	F	Sig.
Males	12	0.00	0.00			4.00	7.82			0.00	0.00			0.00	0.00			3.143	0.035 *
Females	18	0.00	0.00	-	-	4.67	7.29	0.238	0.816	0.00	0.00	-	-	0.00	0.00	-	-	7.372	0.000 **
Total	30	0.00	0.00			4.40	7.38			0.00	0.00			0.00	0.00			10.666	0.000 **

No significant= P value > 0.05. *(Significant)= P value < 0.05. **(Highly significant)= P value < 0.01.

Table (4) showed the correlation coefficient between IL-1β and TNF-α at T0, T1, T2 and T3. Concerning T0, the relation between IL-1β and TNF-α was highly significant correlation in positive direction for the total sample, males and females (P <0.01). Similar findings were reported among T1, T2 and T3.

Table 4. Correlation coefficient (r) between means of IL-1β and TNF-α among samples

		IL-1β and TNF-α							
		Baseline (T0)		After 1hour (T1)		After 1 week (T2)		After 2 week (T3)	
Gender	No.	r	P-Value	r	P-Value	r	P-Value	r	P-Value
Males	12	0.95	0.00**	0.83	0.00**	0.89	0.00**	0.89	0.00**
Females	18	0.95	0.00**	0.75	0.00**	0.94	0.00**	0.92	0.00**
Total	30	0.90	0.00**	0.73	0.00**	0.89	0.00**	0.87	0.00**

**** (Highly significant = P value < 0.01).**

Table (5) showed the correlation coefficient between IL-1β and CRP at T0, T1, T2 and T3. Concerning T0, T2, and T3, the data of the present study showed absence of correlation between IL-1β and CRP. Concerning at T1, the relation between IL-1β and CRP was highly significant correlation in positive direction for the total sample (P <0.01). While among males and females the relation between IL-1β and CRP was significant corelation in positive direction (P <0.05).

Table (6) reported the correlation coefficient between TNF-α and CRP at T0, T1, T2 and T3. Concerning the T0, T2 and T3, the data of the present study showed absence of correlation between TNF-α and CRP. Concerning time (T1), the relation between TNF-α and CRP was highly significant correlation in positive direction for the total sample (P <0.01), similarly the relation between TNF-α and CRP was highly significant in positive direction for the females (P <0.01), while for the males the relation between TNF-α and CRP was significant correlation in positive direction (P <0.05).

Table 5. Correlation coefficient (r) between means of IL-1β and CRP among samples

		IL-1β and CRP							
		Baseline (T0)		After 1hour (T1)		After 1 week (T2)		After 2 week (T3)	
Gender	No.	r	P-Value	r	P-Value	r	P-Value	r	P-Value
Males	12	.	-	0.69	0.01*	.	-	.	-
Females	18	.	-	0.61	0.01*	.	-	.	-
Total	30	.	-	0.60	0.00**	.	-	.	-

*(significant correlation) = P value < 0.05. **(highly significant correlation) = P value < 0.01.

Table 6. Correlation coefficient (r) between means of TNF-α and CRP among samples

		TNF-α and CRP							
		Baseline (T0)		After 1hour(T1)		After 1 week (T2)		After 2 week (T3)	
Gender	No.	r	P-Value	r	P-Value	r	P-Value	r	P-Value
Males	12	.	-	0.69	0.01*	.	-	.	-
Females	18	.	-	0.77	0.00**	.	-	.	-
Total	30	.	-	0.62	0.00**	.	-	.	-

*(significant correlation) = P value < 0.05. **(highly significant correlation) = P value < 0.01.

DISCUSSION

Orthodontic tooth movement is a biological process of bone remodeling, involving an acute inflammatory response in the periodontal tissues, characterized by increasing in vascular permeability and cellular infiltration of leukocytes [2, 13]. Some cytokines such as IL-1β, TNF-α play important role in regulation of bone remodeling during orthodontic tooth movement, through recruitment of osteoclast precursors from the circulation, maturation and activation, in addition they promote osteoclast formation and activation [26-28]. The result of the present study showed that the mean value of salivary level IL-1β and TNF-α were elevated to reach the peak at T1 following placement of the orthodontic appliance during the study periods followed by declined at T2 then T3. This elevation at the 1 hour might be caused by an early upregulation of chemotactic activities directly after continuous mechanical force application and this in agreement with previous findings [5,7-8,29-30]. While disagreement with other studies [6,31] who were demonstrating no change in the IL-1β and TNF-α at the (1hour) in the GCF in orthodontic tooth movement, this may be due to the variations in the study design, and these differences exist in terms of sample size, patients' gender, age and salivary sample type, when many of these studies did not included these factors in the multivariate analysis. Concerning to the declining the level of the cytokines at the T2 and T3 of this study might be to the activity of the anti- inflammatory cytokines such as IL-10 which is known immune regulatory functions, including suppression of the proinflamatory cytokines and stimulation that play a role in bone resorption and periodontal tissue destruction [32]. The present study found no significant differences between males and females, this in agreement with Serra et al. [33] who found no differences in enzymztic activity during orthodontic tooth movement between males and females. The result of the present study revealed synergistically correlation between the IL-1β and TNF-α at all the period times of this study (T0, T1, T2, and T3), this in agreement with Dinarello [34].

Several studies found that salivary CRP may largely reflect local inflammation in the mouth [35-36], adapted to these studies the salivary level of CRP were measured in the present study. The results of this study illustrated the highest level of the CRP at the (T1) with non-significant differences between males and females. Concerning to the correlation between (IL-1β and CRP) and (TNF-α and CRP), there were significant correlation in positive direction at the (T1), this can be rationalize by the transport of the cytokines (IL-1β and TNF-α) from the gingival sites into the systemic circulation which stimulate the hepatocytes in the liver to produce CRP and increase their level, this in agreement with the previous studies [15-17]. The normal of the CRP at the T0 indicated that the patients involved in this study were healthy. In additional, normal of the CRP at the T2 and T3 can be rationalized by decreasing and cease the stimulation effect of the bone resorption, this in agreement with Haheim [37] who demonstrated that the production of CRP related to the stimulation, that the level of CRP falls rapidly when the stimulation is ceased. The result of the present study shown no significant differences between males and females; this

finding come in agreement with the finding of Serra et al. [33] who found that between males and females there was no differences in enzymatic activity during orthodontic tooth movement.

REFERENCES

1. Davidovitch Z. Tooth movement. Critical Reviews in Oral Biology and Medicine 1991; (2): 411–50.
2. Krishnan V, Davidovitch Z. Cellular, molecular, and tissue-level reactions to orthodontic force. Am J Orthod Dentofac Orthop, 2006; 129(4): 469–e1-32.
3. Takahashi I, Onodera K, Nishimura M, Mitnai H, Sasano Y, Mitani H. Expression of genes for gelatinases and tissue inhibitors of metalloproteinases in periodontal tissues during orthodontic tooth movement. J Mol Histo 2006; 37(8-9): 333–42
4. Garlet TP, Coelho U, Repeke CE, Silva JS, Cunha Fde Q, Garlet GP. Differential expression of osteoblast and osteoclast chemmoatractants in compression and tension sides during orthodontic movement. J PMC 2008; 42(3):330–5.
5. Grieve WG, Johnson GK, Moore RN, Reinhardt RA, Dubois LM. Prostaglandin E and interleukin-1 B levels in the gingival crevicular fluid during human orthodontic tooth movement. Am J Orthod 1994; 105(4):369-74.
6. Uematsu S, Mogi M, Deguchi T. Interleukin (IL)-1 beta, IL-6, tumor necrosis factor-alpha, epidermal growth factor, and beta 2-microglobulin levels are elevated in gingival crevicular fluid during human orthodontic tooth movement. J Dent Res 1996; 75(1): 562-7.
7. Giannopoulou C, Dudic A, Kiliaridis S. Pain discomfort and crevicular fluid changes induced by orthodontic elastic separators in children. J Pain 2006; 7(5): 367-76.
8. Filiz AK, Nihal H, Guvenc B, Mehmet D, Tuba TY. TNF-α, IL-1β and IL-8 levels in tooth early leveling movement orthodontic treatment. Journal of International Dental and Medical Res 2010; 3(3):116-121.
9. Sandy JR, Farndale RW, Meikle MC. Recent advances in understanding mechanically induced bone remodeling and their relevance to theory and practice. Am J Orthod Dentofac Orthop 1993; 103(3): 212-22.
10. Azuma Y, Kaji K, Katogi R, Takeshita S, Kudo A. Tumor necrosis factor-alpha induces differentiation of and bone resorption by osteoclasts. J Biol Chem 2000; 275(7): 4858-64.
11. Alhashimi N, Frithiof L, Brudvik P, Bakhiet M. Orthodontic tooth movement and de novo synthesis of pro-inflammatory cytokines. Am J Orthod Dentofac Orthop 2001; 119(3): 307-312.
12. Başaran G, Ozer 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(1): 7.e1-6.
13. 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. (IVSL).
14. Karacay S, Saygun I, Bengi AO, Serdar M. Tumor necrosis factor-alpha levels during two different canine distalization techniques. Angle Orthod 2007; 77(1):142-7. (IVSL).
15. Ide M, Jagdev D, Coward P, Crook M, Barclay G, Wilson R" the short-term effects of treatment of chronic periodontitis on circulating levels of endotoxin, C-reactive protein, tumor necrosis factor alpha, and interleukin-6". J Periodontal 2004; 75(3): 420-8.
16. Gabay C. Interleukin-6 and chronic inflammation. Arithritis Res 2006; 8(1): 2-3.
17. Nibali L, D'Aiuto F, Griffiths G, Patel K, Suvan J, Tonetti M. Severe periodontitis is associated with systemic inflammation and a dysmetabolic status: a case control study. J Clin Periodontal 2007; 34(11): 931-7.
18. Paraskevas S, Huizinga J, loos B. A systematic review and meta-analysis on c-reactive protein in relation to periodontitis. J Clin periodontal 2008; 35(4): 277-90.
19. Takahashi T, Anzai T, Kaneko H, Mano Y, Anazai A, Agai T, kohno T, Maekawa Y, Yoshikawa T, Fukuda k, Ogawa S. increased c-reactive protein expression exacerbates left ventricular dysfunction and remodeling after myocardial infarction. Am J Physiol Heart and Circulatory Physiol 2010; 299(6):1795-804.
20. Ren Y, Maltha JC, Van't Hof MA, Von Den Hoff JW, Kuijpers-Jagtman AM, Zhang D. Cytokine levels in crevicular fluid are less responsive to orthodontic forces in adults than in juveniles. J Clin Periodontol 2002; 29(8):757-62.
21. Frodge BD, Ebersole JL, Kryscio RJ, Thomas MV, Miller CS. Bone remodeling biomarkers of periodontal disease in saliva. J Periodontol 2008; 79(10):1913-9.
22. Capelli J Jr, Kantarci A, Haffajee A, Teles RP, Fidel R Jr, Figueredo CM. Matrix metalloproteinases and chemokines in the gingival crevicular fluid during orthodontic tooth movement. Eur J Orthod 2011; 33(6): 705-11.
23. Tenovuo J, Lagerlöf F. Saliva. In Thylstrup A, Fejerskov O (eds). Textbook of clinical cariology 2nd ed. Copenhagen: Munksgard; 1996. pp.17-43.
24. Farsi NMA. Signs of oral dryness in relation to salivary flow rate, pH, buffering capacity and dry mouth complaints. BMC Oral Health 2007; 7:15.
25. Fabrício T.A, Tânia MP, Tálita PM, Evandro NA, Maria CF, Antônio L, Arthur MK, Mauro HNG. Tarcília A. Burning mouth syndrome. Headache 2012; 52(6):1026-34
26. Glantschnig H, Fisher JE, Wesolowski G, Rodan GA, Reszka AA. M-CSF, TNFalpha and RANK ligand promote osteoclast survival by signaling through mTOR/S6 kinase. Cell Death Differ 2003; 10(10):1165-77.
27. Seidenberg AB, An YH. Is there an inhibitory effect of COX-2 inhibitors on bone healing? Pharmacol Res 2004; 50(2):151-6.
28. Yao Z, Xing L, Qin C, Schwarz EM, Boyce BF. Osteoclast precursor interaction with bone matrix induces osteoclast formation directly by an interleukin-1-mediated autocrine mechanism. J Biol Chem 2008; 283(15): 9917-24.
29. Lee KJ, Park YC, Yu HS, Choi SH, Yoo YJ. Effects of continuous and interrupted orthodontic force on interleukin-1 and prostaglandin E2 production in gingival crevicular fluid. Am J Orthod Dentofac Orthop 2004; 125(2):168-77.
30. Dudic A, Killiaridis S, Mombelli A, Giannopoulou C. composition changes in gingival crevicular fluid during orthodontic tooth movement: comparisons

between tension and compression sides. Eur J Oral Sci 2006; 114(5): 416-22.
31. Iwasaki LR, Haack JE, Nickel JC, Reinhardt RA, Petro TM. Human interleukin-1B and Interleukin -1 receptor antagonist secretion and velocity of tooth movement. Archives Oral Biol 2001; 46:185-189.
32. Hirose M, Ishihara K, Saito A, Nakagawa T, Yamada S, Okuda K. Expression of cytokines and inducible nitric oxide synthase in inflamed gingival tissue. J Periodontol 2001; 72(5): 590-7.
33. Serra E, Perinetti G, D'Attilio M, Cordella C, Paolantonio M, Festa F, Spoto G. Lactate dehydrogenase activity in gingival crevicular fluid during orthodontic treatment. Am J Orthod Dentofac Orthop 2003; 124(2): 206-11.
34. Dinarello CA. Interleukin 1 and its biologically related cytokines. Adv Immunol 1989; 44:153-205.
35. Higashi Y, Goto C, Jitsuiki D, et al. Periodontal infection is associated with endothelial dysfunction in healthy subjects and hypertensive patients. Hypertension 2008; 51(2): 446-453.
36. Giannobile WV, Beikler T, Kinney JS, et al. Saliva is as a diagnostic tool for periodontal disease current state and future directions. J Periodontal 2009; 50: 52-64.
37. Haheim LL, Nafstad P, Olsen I, Schwarze P, Ronningen KS. C-reactive protein variation for different chronic somatic disorders. Scand J Public Health 2009; 37(6): 640-6. Republished 2012; 40(1).