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Evaluation of the Osteocalcin Salivary Levels during Early Stage of Orthodontic Tooth Movement by Using (Fixed Orthodontic Appliance andAcceledent Device)

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Abstract
Background:*AcceleDent vibratory device can increase the rate of tooth movement when used in conjunction with conventional Orthodontic force. during OTM their will lead to stimulate cell of periodontium to release many mediators like inflammatory mediators as cytokines in addition to proteins such as Osteocalcin into the oral fluid as (saliva & gingival crevicular fluid).*
Aim of this study *is to evaluate the influences of orthodontic tooth movement on the salivary level of OC during the early stage of OTM by using Acceledent vibration device in addition to fixed orthodontic appliance.*
Methods:*Thirty adult Iraqi patients with age range (18-23) years olddiagnosed as CL I dental malocclusion.The subjects were equally and randomly allocated to two treatment groups. The first 15 subjects continued with the standard treatment (control group), while the second group received Acceledent device (intervention group) in addition to the fixed orthodontic appliance. Unstimulated whole saliva in both groups were measured at baseline (before treatment), one hour after treatment and then one, two and three weeks after treatment.*
Results:*both groups (control and intervention) show a statistically significant ($p<0.001$) mean increase in salivary osteocalcin after each of the four time intervals compared to the pretreatment level. In addition the intervention group was associated with an extra increase in salivary osteocalcin compared to control treatment after one hour and after 3 weeks of treatment with statistical significant ($p<0.001$).*
Conclusion: *The use of acceledent device in orthodontic treatment induced a markedly increase the salivary level of OC that considered as a specific markers of bone turnover.*
Keywords:*Acceledent, Orthodontic tooth movement, Osteocalcin*

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I. Introduction

In clinical orthodontic treatment is based on applying a force system to an individual or groups of teeth, that induced periodontal ligaments (PDL) and alveolar bone remodeling[(1)]. Following mechanical force , an inflammatory response in the periodontal tissues results in local synthesis and release of various molecules such as (cytokines, growth factor, and colony stimulating factors) that provide a best microenvironment for tissue deposition and resorption [(2)] .During bone remodeling, sufficient quantity of variety of substances are involved and produced by the PDL cells and diffused in the GCF [(3)]**,** which is related to one hand on the released of inflammatory mediators such as (prostaglandin E2, il-1beta, and on the other hand to the production of neuropeptides such as substance P and IL-1beta.that participate in initiate bone resorption [(4, 5)].Osteocalcin is the main non-collagen protein of bone matrix, which is primarily synthesized by osteoblasts although osteocalcin considered as a specific markers for bone formation when bone formation and resorption are uncoupled, but can be considered as a valid markers for bone turnover when formation and resorption are coupled [(6)].Studies found Elevated serum osteocalcin levels have been found during periods of rapid bone turnover, such as in osteoporosis and multiple myeloma and during fracture repair. Therefore, studies reported the present of OC at the GCF of periodontal disease patients , and other study found the elevation GCF OC concentration were associated with high rates of bone turnover[(7,8)].Previous studies, examine the OC levels at GCF during orthodontic tooth movement during various stages of treatments, while others try to assess the influence of orthodontic tooth movements on the composition of OC in GCF during the various stages of orthodontic tooth movements[(9,10)], there is no previous study try to evaluate the OC levels in saliva in response of using Acceledent vibration device with fixed orthodontic appliance , so this study considered the first

Iraqi study to evaluate the salivary levels of OC during the early stage of orthodontic tooth movements by using Acceledent vibration device.This study aim to evaluate the influences of orthodontic tooth movement on the salivary level f during the early stage of OTM by using Acceledent vibration device in addition to fixed orthodontic appliance.

II. Material & Methods

2. 1Dental materials

1. Diagnostic dental instruments (dental mirrors, dental probes, dental tweezers)
2. Stainless steel Bracket, 0.018 x 0.022 inch (Ortho Technology Company/ U.S.A)
3. 0.014 inch, light, round Nickel Titanium arch wire (Ortho Technology Company /U.S.A)
4. Molar tubes for both maxillary and mandibular molars.
5. Light cure composite and bonding (Orthotechnology).

2.2 Laboratory materials:

1. Disposable test tube
2. Eppendroffs tubes (1 ml)
3. Eliza kits
4. Glass Pasteur pipettes

III. Subjects And Methods

Thirty adult Iraqi patients with age range (18-23) years old were selected from private dental clinic seeking for orthodontic treatments and diagnosed as CLI dental malocclusion. The participants were divided into two groups: control group (15 participants): treated with fixed orthodontic appliance only, intervention group (15 participants): treated with fixed orthodontic appliance and Acceledent vibration device with frequency vibration (30 Hz) to the teeth for approximately 20 minutes per day.

3. 1The inclusion criteria for the participants were

1. Iraqi orthodontic patients,
2. No history of systemic disease such as osteoporosis, gastrointestinal diseases related to nutrition and mineral metabolism, endocrine diseases, immunological disorders, connective tissue diseases that may affect the host's periodontal status and bone metabolism or that would require pre-medication for monitoring or treatment procedures (e.g. heart conditions, joint replacements, hormonal or bisphosphonate anti-resorptive therapies[11, 12].
3. No history of previous orthodontic and facial surgical treatments,
4. No smoking.
5. The Pregnancy and lactation patients and Patients with (dental carries, pulp pathology, and periodontal disease) were excluded.

Additionally, Informed consent were obtained from the patient Following that all the participants were given repeated oral hygiene instructions (OHIs), including the correct use of tooth and interdental tooth brush prior placement the orthodontic appliance for at least three week to ensure control of oral hygiene following that fixed orthodontic appliances were bonded for both arch from right second premolar to left second premolar and bonding the Molar tube for maxillary and mandibular first molar right and left, then were ligated by ligature wire.For intervention group in addition to fixed orthodontic appliance the patient were instructed for using acceledent vibration device and activated it once daily 20 minutes.

3.2 Salivary Sample

Unstimulated whole saliva were collected into sterile plastic tubes at different stages (T0: baseline before placement orthodontic appliances, T1: one hour after placement of orthodontic appliance, T2: after one week, T3: after two week, T4: after three week)under standardized conditions, following the instructions cited [13, 14].About 2 ml of the whole unstimulated saliva samples were collected in restful and quiet circumstances for bothgroup, then the samples weresaved in a small cooling box after collection to stop the growth of bacteria until taken to the laboratory for centrifuged and stored at the same day. After that the samples were centrifuged at 3000 rpm for 10 min, the clear supernatant layer were collected by pasture pipette, then distributed the collected clear supernatant into eppendroffs tubes (for osteocalcin), 1 ml in each eppendroffs tubes, then the samples were stored at -20ºc in a deep freeze until processed[15]. Then data were analyzed by using SPSS statistical software program (version 10 for window, SPSS).

IV. Results

The results presented in this study were based on the analysis of 30 adults needing orthodontic treatment with fixed appliance. The subjects were equally and randomly allocated to two treatment groups. The first 15 subjects continued with the standard treatment (control group), while the second group received Acceledent device (intervention group) in addition to the fixed orthodontic appliance. Both groups were measured at baseline (before treatment), one hour after treatment and then one, two and three weeks after treatment. No loss to follow up was experienced in this clinical trial.

Table 1: Intervention-control group difference in mean age.

	Study group		
	Control group (fixed orthodontic appliance)	**Intervention group (Acceledent device)**	
Age (years)			0.81[NS]
Range	(18 to 23)	(18 to 23)	
Mean	20.2	20.0	
SD	1.8	1.8	
SE	0.5	0.5	
N	15	15	

Table 2: Gender distribution by study group.

	Study group			
	Control group (fixed orthodontic appliance)		**Intervention group (Acceledent device)**	
	N	**%**	**N**	**%**
Gender				
Female	7	46.7	7	46.7
Male	8	53.3	8	53.3
Total	15	100.0	15	100.0

As shown in table 1, no obvious or statistically significant difference in mean age was observed between the two groups (20 years of age for each). The age ranged between 18 to 23 years. In addition the gender distribution was equal between the two groups, table 2. It is obvious from the previous discussion that the randomization process was effective in cancelling the differences between the two groups with respect to known and unknown confounders.

Table 3: The difference between intervention and control groups in salivary osteocalcin level at selected time intervals.

Salivary osteocalcin (pg/micro-liter)	at baseline (pretreatment)	after one hour of treatment	changes after one hour of treatment compared to pretreatment	after one week of treatment	changes after one week of treatment compared to pretreatment	after two weeks of treatment	changes after two weeks of treatment compared to pretreatment	after three weeks of treatment	changes after three weeks of treatment compared to pretreatment
Control group (fixed orthodontic appliance)									
Range	(2.06 to 4.7)	(2.62 to 6.16)	(0.55 to 1.46)	(4.8 to 10.9)	(2.74 to 6.2)	(5.3 to 12.08)	(3.22 to 7.38)	(5.51 to 12.81)	(3.45 to 8.11)
Mean	3.25	4.18	0.93	7.66	4.42	8.37	5.13	8.82	5.57
SD	0.87	1.13	0.28	2.00	1.19	2.22	1.36	2.38	1.51
SE	0.22	0.29	0.07	0.52	0.31	0.57	0.35	0.61	0.39
N	15	15	15	15	15	15	15	15	15
P (paired t-test)			**<0.001**		**<0.001**		**<0.001**		**<0.001**
Intervention group (Acceledent device)									
Range	(2.34 to 5.1)	(3.25 to 7.3)	(0.91 to 2.2)	(11.14 to 24.19)	(8.8 to 19.09)	(17.15 to 37.35)	(14.81 to 32.25)	(22.76 to 57.48)	(20.42 to 52.38)
Mean	3.64	5.19	1.55	17.27	13.63	26.66	23.02	36.05	32.41
SD	0.84	1.21	0.37	3.97	3.13	6.16	5.32	9.24	8.44
SE	0.22	0.31	0.10	1.03	0.81	1.59	1.37	2.39	2.18
N	15	15	15	15	15	15	15	15	15
P (paired t-test)			**<0.001**		**<0.001**		**<0.001**		**<0.001**
Effect of intervention compared to control									
Difference in mean	0.395		0.618		9.210		17.889		26.840
Cohen's d	0.46		1.88		3.89		4.6		4.43
P	0.87[NS]		0.16[NS]		0.002		<0.001		<0.001

As shown in table 3, the mean salivary osteocalcin at baseline (pretreatment level) was not significantly different between the two treatment groups. Both treatment groups showed a statistically significant mean increase in salivary osteocalcin after each of the four time intervals compared to the pretreatment level. This raise in salivary osteocalcin was of a higher magnitude in the intervention group when compared to control group at each of the follow up interval.

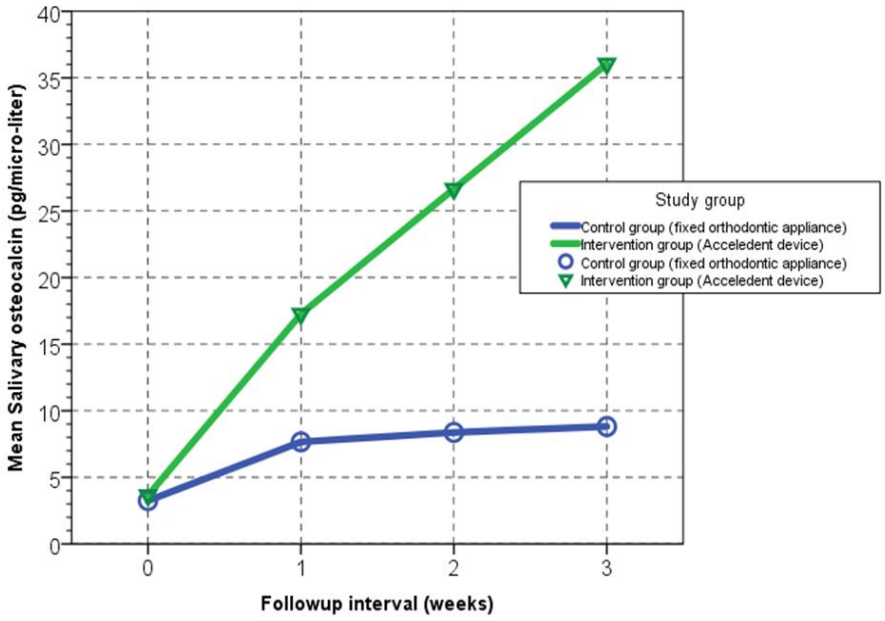

Figure 1: Line graph showing the mean salivary osteocalcin (pg/uL) in the two study groups after selected time intervals from treatment inception.

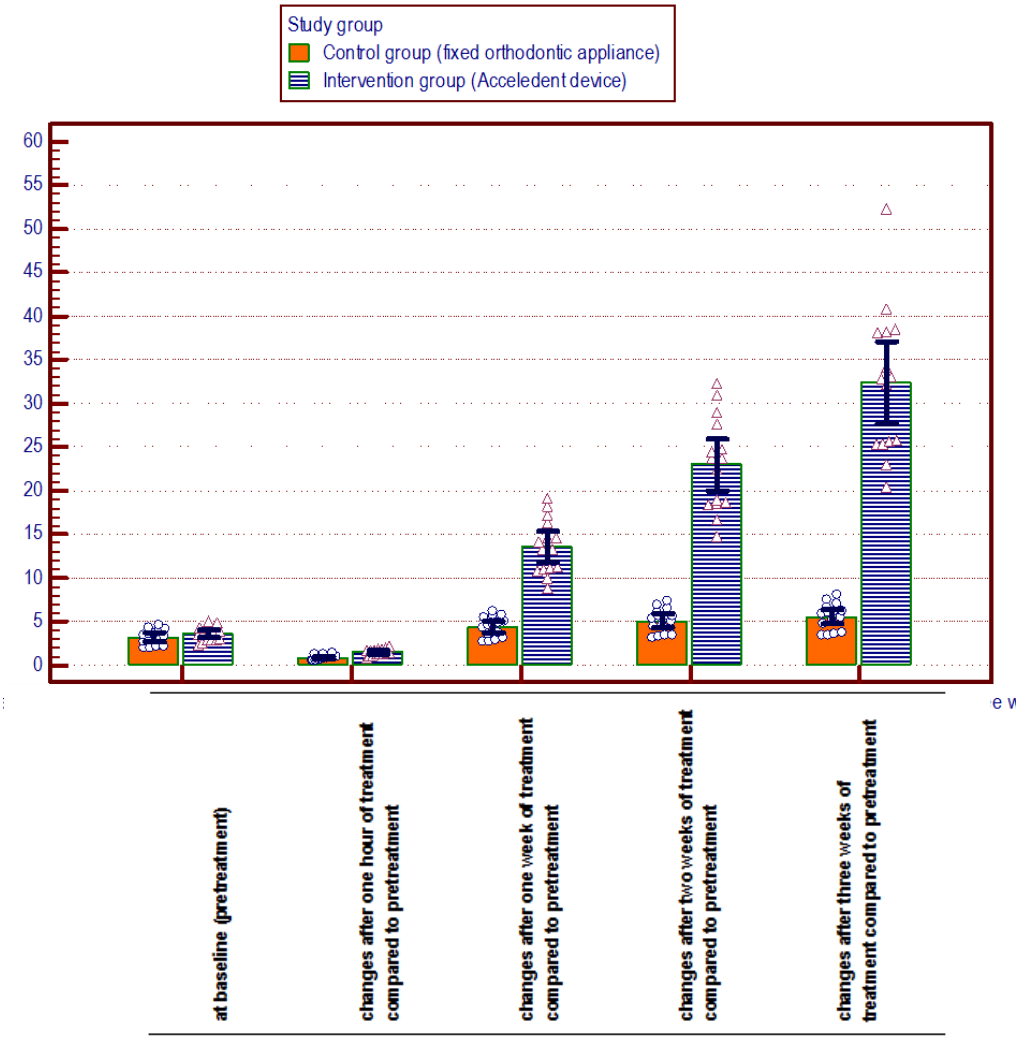

Figure 2: Dot diagram with error bars showing the mean (with its 95% confidence interval) salivary osteocalcin (pg/uL) in the two study groups after selected time intervals from treatment inception.

In addition, the gap between the two groups kept widening with time as shown in figure 1&2 and the increasing value of Cohen's d effect size measure. The intervention was associated with a statistically higher mean change in salivary osteocalcin after each time interval compared to its pretreatment level. The effect of the intervention compared to control standard treatment was evaluated as a large effect (since Cohen's d is larger than 0.8) in each of the four follow up intervalsAs early as after one hour of treatment the salivary osteocalcin increased by a mean of 1.55 pg/uL in the intervention group, which surpassed that observed with control group (0.93 pg/uL) by a mean of 0.618 pg/uL. This extra raise in salivary osteocalcin gets larger as the time of follow up moves from 1 hour to 3 weeks (an extra 28.84 pg/uL).

Table 4: The effect of intervention adjusted for age and gender on changes in salivary osteocalcin (pg/µl) after one hour of treatment compared to baseline as the dependent variable**.**

	Unstandardized Partial Regression Coefficient	**P**
(Constant)	1.40	0.06[NS]
Male gender compared to female	-0.18	0.15[NS]
Age (years)	-0.02	0.59[NS]
Intervention group (Acceledent device + fixed orthodontic appliance) compared to control group (fixed orthodontic appliance)	0.61	<0.001

R^2=0.53

P (Model) < 0.001

Table 4 shows the age and gender adjusted effect of intervention (Acceledent device+ fixed orthodontic appliance) compared to control group (fixed orthodontic appliance) on salivary osteocalcin. The Acceledent device was associated with an extra 0.61 pg/uL increase in salivary osteocalcin compared to control treatment after one hour of treatment. The model was statistically significant and able to explain 53% of variation in the outcome variable.

Table 5: The effect of intervention adjusted for age and gender on changes in salivary osteocalcin (pg/µl) after three weeks of treatment compared to baseline as the dependent variable**.**

	Unstandardized Partial Regression Coefficient	**P**
(Constant)	7.90	0.56[NS]
Male gender compared to female	-3.09	0.18[NS]
Age (years)	-0.03	0.96[NS]
Intervention group (Acceledent device + fixed orthodontic appliance) compared to control group (fixed orthodontic appliance)	26.83	<0.001

R^2=0.85

P (Model) < 0.001

Table 5 shows the age and gender adjusted effect of intervention (Acceledent device+ fixed orthodontic appliance) compared to control group (fixed orthodontic appliance) on salivary osteocalcin after three weeks of treatment compared to pretreatment level. The Acceledent device was associated with an extra 26.86 pg/uL increase in salivary osteocalcin compared to control treatment after three weeks of treatment. The model was statistically significant and able to explain 85% of variation in the outcome variable.

V. Discussion

The orthodontic treatments is based on the principles that if prolonged pressure is applied on the tooth that initiate biochemical and structural responses in a variety of cell type that lead of tooth moving as the surrounding bone remodelling[16-17]. So the analysis of salivary samples might be a good ideas of evaluate the ongoing biochemical and structural response that related with bone remodeling. Additionally during active bone resorption OC and its fragments released in GCF [18-20], Corresponding to that, salivary levels of OC was evaluated in this study.Due to the diagnostic bio-fluid (saliva and blood serum) contain similar proteins and RNA, so the diagnostic medium in this study was saliva instead of (blood serum because, its easily collected and noninvasive than serum which makes the procedure more acceptable to patients and more conducive to a stress-free appointment [21].

Generally, Osteocalcin is a non-collagenous matrix protein, located at the alveolar bone, root cementum and subpopulations of periodontal ligament cells. Both interventional and control groups showed a statistically significant increase in salivary osteocalcin after each of the four time intervals compared to the pre-treatment level this in agreement with previous study (Sarah & Sukumaran, 2011)[22], this might indicate the roles of OC in bone resorption and formation by regulating maturation of hydroxyapatite and osteoclast recruitment which participate in

bone remodelling, However, the molecular weight of osteocalcin is small (-6Da) in comparing with many other bone proteins, that may not require extensive cleavage for detachment from the bone matrix [23-25].

Also, results reported statistical significance raise in salivary osteocalcin was of a higher magnitude in the intervention group when compared to control group at each of the follow up interval this might explain the functional role of acceledent device in addition to orthodontic fixed appliance in stimulation of bone resorption that lead to release of OC into the bio-fluid from the matrix[26-27].So our results explain the role of acceledent device in stimulation the release of salivary biochemical markers of bone resorption that reflex the acceleration of bone resorption in agreement with previous study (Zaid et al, 2015)[27].As a conclusion the use of acceledent device in addition to fixed orthodontic appliance was found to be effectively and markedly increase the salivary level of OC, therefore OC is considered as a specific markers of bone turnover

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