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## Changes in Osteocalcin Levels During Orthodontic Tooth Movement in Periodontally Affected Mandibular Anterior Teeth

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**Aim:** This study aimed to investigate the changes in Gingival Crevicular Fluid Osteocalcin (GCF OS) levels during the canine retraction phase in adult patients with periodontally affected teeth.

**Material and Methods:** A sample of 22 orthodontic adult female patients (aged 25-35 years) was included in the present study. The patients had a history of periodontal problems and were indicated for orthodontic intervention with a plan for lower first premolar extraction. The sectional arch technique and temporary anchorage devices were implemented for canine retraction. GCF OC samples were collected from the deepest periodontal pocket at T0, before orthodontic treatment; at T1, after 3 months of canine retraction; and at T3, 6 months after canine retraction. The collected samples were analyzed using an enzyme-linked immunoassay (ELISA).

**Results:** The registered average GCF OC concentrations at T0, T1 and T2 were 34.31, 34.69 and 35.30 ng/ml, respectively. The change in GCF osteocalcin concentrations from T0 to T1 showed a marginal increase of .370 ng/ml in GCF. A considerable difference was observed between T1 and T2 (0.616 ng/ml of GCF). The overall change in the GCF osteocalcin concentration between T0 and T2 was 0.986 ng/ml GCF. A pairwise comparison among the three studied observation periods revealed a statistically nonsignificant difference ( $p>0.05$ ).

**Conclusions:** Adult orthodontic treatment has no serious harmful effects on the periodontium as this study showed a nonsignificant increase in the gingival clavicular fluid Osteocalcin concentration level through the 6-month follow-up period, which may be positively correlated with the improvement in periodontal parameters.

**Keywords:** Adult orthodontics, periodontally affected teeth, osteocalcin.

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## Introduction

Orthodontic tooth movement is achieved through the application of controlled therapeutic forces that elicit bone remodeling in the direction of the intended movement. These forces induce signaling pathways mediated by various osteogenic proteins, including OPN, bone sialoprotein, Osterix, and osteocalcin. Nonetheless, teeth subjected to orthodontic forces should have healthy periodontium to avoid any undesirable periodontal side effects.<sup>1,2</sup>

Recently, the number of adult patients seeking orthodontic treatment has increased exponentially, and the number of patients is expected to increase in the upcoming years (3). Orthodontic treatment for adult patients has two challenges. The first challenge is age-related changes in the periodontium, which include a reduced number of fibroblasts, elastic fibers, and organic matrix production. Cortical bone becomes denser, spongy bone decreases in size, and the bone structure changes from a honeycomb to a lace network.<sup>4-7</sup> The second challenge is determining the pathological conditions that are representative of periodontitis. Periodontitis is defined as an irreversible, chronic, multifactorial, immune-inflammatory condition that leads to progressive destruction of soft and hard tooth-supporting structures. The cardinal clinical presentations of periodontitis include attachment loss, excessive bony and cementum destruction, and the formation of deep periodontal pockets. Apical displacement of the crestal bone influences tooth movement in adults, and more attention should be given to the utilization of orthodontic biomechanics.<sup>8-11</sup>

The pathophysiology of periodontitis involves a complex, multifactorial, reactive immune cascade that occurs because of the interaction between the host immune system and bacterial pathogens. Activation of macrophages and monocytes occurs after

pathogen exposure.<sup>12-16</sup> This exposure leads to an influx of certain proinflammatory mediators, such as cytokines/chemokines (interleukins tumor necrosis factor alpha), prostaglandins, and matrix metalloproteinases. These inflammatory mediators provoke the initiation and progression of destructive inflammatory processes.<sup>17-20</sup> Biological biomarkers are objectively measured surrogate endpoints that are found in gingival crevicular fluid, blood serum, saliva, or any other body fluids and act as mirrors for observing normal physiological events and diagnosing pathological processes, in addition to evaluating therapeutic interventions.<sup>21-25</sup>

Osteocalcin (Gla- protein) is a nonmineralized, low-molecular-weight bone protein with high affinity for binding with calcium. It is synthesized and secreted by osteoblasts, odontoblasts, and chondrocytes and occurs in two physiological states the inactive carboxylated state and the free, active uncarboxylated state that circulate in serum/plasma.<sup>26-28</sup> The osteocalcin (OC) biomarker is generally considered an indicator of bone formation; however, in periodontitis, OC recruits osteoclasts to the site of bone degradation and promotes their differentiation into active osteoclasts. This shift in function might be attributed to the disturbance of bone homeostasis due to the increased resorption rate.<sup>28-32</sup>

The same phenomenon was observed during orthodontic tooth movement: osteocalcin has a chemotactic effect on osteoclasts on the pressure side during orthodontic force application, and increased OC levels in the GCF are an indication of active tooth movement. An experimental study reported that the injection of osteocalcin at the pressure side might accelerate tooth movement in rats.<sup>33-35</sup>

According to the literature, no study has investigated the changes in GCF OC levels during orthodontic treatment in

periodontally compromised patients. Therefore, the aim of the current study was to answer the following research question: “What is the change pattern in the GCF OC concentration in adult orthodontic patients with compromised periodontal conditions at different time points during the canine retraction phase?”

## Material and Methods

### PIOTS

**Population:** Adult orthodontic female patients with controlled periodontally compromised conditions.

**Intervention:** Orthodontic canine retraction using a power arm.

**Outcomes:** Changes in GCF OC levels.

**Time of observation:** at T<sub>0</sub> (before orthodontic tooth movement), T<sub>1</sub> (3 months after the start of canine retraction) and T<sub>2</sub> (6 months after the start of canine retraction).

**Study design:** Single-arm interventional study.

### Hypothesis

The null hypothesis posited that there is no difference in Osteocalcin levels during mandibular canine retraction in female patients with periodontally affected teeth.

### Study Design

A total of 22 adult female patients (aged 25-35 years) were included in this study. Patients were recruited from outpatient clinics in the Orthodontic Department of the Faculty of Dental Medicine, Al-Azhar University, Cairo, Egypt. The purpose and procedures were thoroughly explained to all subjects prior to participation, and all participants provided written informed consent in accordance with the Helsinki Declaration. The study protocol was approved by the Research Ethics Committee (REC) of the faculty of Dental Medicine (code REC-OR-22-03). Moreover, this study was registered in ClinicalTrials.gov

(NCT06102616) October 26, 2023. Complete medical and dental histories were taken from all the subjects.

### Inclusion criteria

All the included patients were indicated for therapeutic orthodontic extraction of the lower first premolars with subsequent canine retraction to alleviate lower anterior crowding (>7 mm according to Little's irregularity index)<sup>37</sup>. None of the included subjects had a history of systemic disease or medications that might interfere with orthodontic tooth movement. Patients with severe medical disorders, including diabetes mellitus and immunological disorders, as well as smokers, were excluded from the study. Pregnant and lactating females and those taking oral contraceptive drugs were also excluded from the study.

### Sample Size Calculation

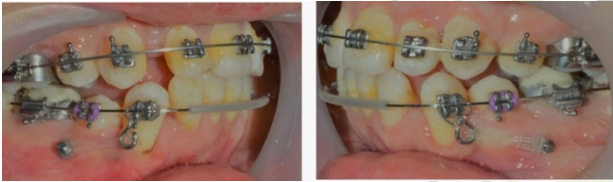
The sample size was calculated based on a previous study<sup>33</sup> using PASS 11.0 (NCSS statistical software, Kaysville, Utah) for a standard deviation of 5 (standard value of 1.96); a sample size of 22 patients was needed, using a study power test of 80% with a dropout rate of 20%.

### Intervention

For every patient, a comprehensive periodontal evaluation with a full periodontal chart was performed, in addition to the essential orthodontic records, which included extraoral and intraoral photographs, lateral cephalometric radiographs, panoramic radiographs and an orthodontic study model.

**Periodontal Phase:** The included patients received conventional periodontal therapy involving full-mouth supra- and subgingival scaling using a hand instrument and ultrasonic scalers. Afterwards, strict follow-up and oral hygiene instructions were given.

**Orthodontic treatment phase:** A fixed orthodontic appliance, 0.022-inch Roth prescription brackets (Atlas Mini, Dynaflex, Missouri, USA), was used and bonded with adhesive (Green-glo Ormco Co., Glendora, CA, USA). The canine bracket was modified by welding an 8 mm power arm onto the bracket base. The sectional arch technique was implemented by levelling and aligning the posterior segment while bypassing the incisors until 0.017"x0.022" stainless steel wires were reached. Temporary Anchorage Devices TADs (Absoanchor Miniscrew, Dentos Inc., Taegu, Korea; 1.6 by 8 mm, self-drilling) were placed between the mandibular second premolar and mandibular first molar for anchorage. (Figure 1)



**Figure 1: Canine power arms and TADs.**

Immediately after the extraction of the mandibular first premolar, canine retraction was performed using a force of 100 g produced by a memory elastomeric chain<sup>(126)</sup> (American Orthodontics® (AO®)) extended from TADs to the soldered vertical power arms of the canine brackets. The force magnitude was measured using a digital force gauge. Patients were recalled every 3 weeks to check the magnitude of the delivered force and ensure constant force application. (Figure 2)



**Figure 2: Different stages of lower canine retraction. A: At T0 immediately after canine extraction. B: After 3 months at T1. C: After 6 months at T2.**

**GCF processing:** Samples of GCF osteocalcin were collected from the greatest pocket depth for each mandibular canine.

The supragingival biofilm was carefully removed using sterile cotton pellets, and the sites were gently dried with an air syringe to avoid contamination with saliva. The GCF was collected using a standard paper point that was inserted into the isolated periodontal pocket for 30 seconds. Paper points visually contaminated with blood were discarded. The paper points with GCF samples were stored frozen at -80°C. Osteocalcin levels were evaluated via an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. The results are expressed as concentrations (ng/ml of GCF).<sup>30-35</sup> (Fig. 3)



**Figure 3: Paper points with GCF samples**

Osteocalcin was extracted from the paper point by adding 100 ml of phosphate-buffered saline to the Eppendorf tube. The mixture was vortexed and subsequently centrifuged for 10 min at 3000×g. The supernatant was used for estimation of osteocalcin. For the quantitative determination of the human osteocalcin concentration in GCF, a Human Osteocalcin Elisa Kit provided by Shanghai Korain Biotech Co.,China, was used. The standard curve was generated by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and the best fit curve was drawn through the points on the graph. These calculations can be performed with computer-based curve-fitting software, and the best fit line can be determined via regression analysis.<sup>30-36</sup>

## Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA) for comparisons between repeated measurements at  $t_0$ ,  $t_1$  and  $t_2$ . P values  $<0.05$  indicated statistical significance. All the statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 23.

## Results

In the present study, the average GCF OC concentrations at  $T_0$ ,  $T_1$  and  $T_2$  were 34.31, 34.69 and 35.30 ng/ml, respectively (Table 1).

The change in GCF osteocalcin concentrations from  $T_0$  to  $T_1$  showed a marginal increase of .370 ng/ml in GCF. Moreover, a considerable difference was observed between  $T_1$  and  $T_2$  (0.616 ng/ml of GCF). The overall change in the GCF osteocalcin concentration between  $T_0$  and  $T_2$  was 0.986 ng/ml of GCF (Table 2 and Fig. 4).

A repeated-measures ANOVA with a Greenhouse–Geisser correction determined that the mean osteocalcin concentrations were significantly different at  $T_0$ ,  $T_1$  and  $T_2$  ( $F(1.981, 39.625) = 39.646, p > .05$ ).

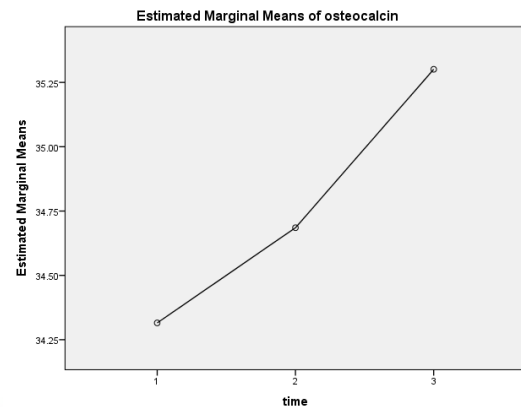


Figure 4: Line chart for changes in the GCF osteocalcin concentration at  $T_0$ ,  $T_1$  and  $T_3$

## Discussion

Orthodontic treatment of adult patients is considered challenging since the prevalence of periodontal diseases among this population is considered the sixth most common disease and may cause tooth loss. The presence of crowding and malocclusion that facilitate plaque accumulation may aggravate periodontal disease progression. Therefore, orthodontic treatment is beneficial for preserving and restoring deteriorating periodontal conditions. However, tooth movement with compromised periodontal support poses great challenges for orthodontists.<sup>4,5</sup>

Fixed orthodontic treatment is considered a lengthy process and, on average, takes 12-36 months. It is associated with adverse outcomes, such as difficulty maintaining proper oral hygiene, which may be detrimental to the teeth and surrounding periodontal tissues. In addition, pain is a common complaint during orthodontic treatment and may lead to weakened masticatory force and poor oral health. Therefore, shortening the treatment duration while controlling pain and gingival inflammation is key to ensuring safe and efficient tooth movement in ortho-perio patients.<sup>5-7</sup>

Table 1: Showing changes of the osteocalcin concentration in (ng/ml of GCF) at  $T_0$ ,  $T_1$  and  $T_2$

Time	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
$T_0$	34.315	1.549	31.084	37.546
$T_1$	34.685	1.532	31.490	37.881
$T_2$	35.301	1.332	32.522	38.080

Table 2: showing the pairwise comparisons among repeated osteocalcin concentration measurements

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.a	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
$T_0$	$T_1$	-.370	2.110	1.000	-5.881	5.141
	$T_2$	-.986	1.559	1.000	-5.059	3.087
$T_1$	$T_0$	.370	2.110	1.000	-5.141	5.881
	$T_2$	-.616	1.355	1.000	-4.157	2.926
$T_2$	$T_0$	.986	1.559	1.000	-3.087	5.059
	$T_1$	.616	1.355	1.000	-2.926	4.157

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

The clinical use of biological markers for diagnosis, prognosis and evaluation of treatment outcomes has been proven to be beneficial. Osteocalcin levels can be used to monitor the progress and effectiveness of orthodontic tooth movement. Increased levels of osteocalcin may indicate active tooth movement, while decreased levels may suggest a plateau or completion of tooth movement. Therefore, Osteocalcin levels can provide valuable information for treatment planning by assessing the levels of osteocalcin, orthodontists can determine the optimal timing and duration of treatment, as well as the need for additional interventions such as low-level laser therapy to accelerate tooth movement. It has been reported that in periodontitis, osteocalcin is a marker of bone formation where bone resorption is greater than formation. GCF osteocalcin levels are more strongly associated with bone turnover in the periodontium than serum or saliva levels. Monitoring osteocalcin levels throughout orthodontic treatment can help assess the patient's response to the applied forces. If osteocalcin levels remain low or show minimal changes, it may indicate a lack of response to treatment, prompting the need for adjustments or alternative treatment approaches.<sup>18,20,23,32</sup>

The current study included only adult female patients to rule out sexual dimorphism that might affect the level of osteocalcin expression. The hormonal fluctuations that occur during the menstrual cycle can affect the bone metabolism and remodeling process. By studying a female population, the researchers could better understand the impact of these hormonal changes on the bone remodeling process during orthodontic treatment.<sup>28</sup> The samples were stored at  $-80^{\circ}\text{C}$  to limit the potential effects of proteases from both the host and the bacterium.<sup>29-32</sup>

The biomechanics of the sectional arch technique were implemented in the present

study because of the ability to allow access to oral hygiene, avoid round tripping (jiggling movement) of the lower incisors and help shorten the treatment duration. An 8 mm canine power arm was used to compensate for the crestal alveolar bone loss that is usually associated with the apical migration of the estimated center of resistance and to ensure, as much as we can, bodily canine retraction. Bodily canine retraction reportedly decreases the incidence of crestal alveolar bone loss due to the even distribution of strains along the pressure side of the periodontal membrane. The use of TADs was necessary due to the amount of space needed to alleviate lower anterior crowding and the compromised periodontal status of the posterior anchor teeth.<sup>20,28</sup>

The present study showed a nonsignificant increase in the GCF OC concentration through from the base line to the first 3 months and at 6-month follow-up period. These results may be positively correlated with the improvement in periodontal parameters. Changes in osteocalcin levels reflect the ongoing bone remodeling process during orthodontic treatment. These results may indicate controlled periodontal bone turnover concomitant with orthodontic therapy. These findings are in accordance with previous studies reporting a marked increase in the level of GCF OC during the active phase of orthodontic tooth movement, with a tendency to decrease at the end of treatment.<sup>33-39</sup> A previous study on healthy periodontium analyzed the levels of osteocalcin in GCF during different stages of orthodontic tooth movement. The study found that there were statistically significant changes in osteocalcin levels on days 7, 14, and 21 after tooth retraction. The peak in osteocalcin activity occurred on day 14, indicating the active tooth movement phase in orthodontic therapy.<sup>33</sup>

Since the observation period of the current study was limited to only 6 months, the declining phase of the GCF osteocalcin was not detected for full canine retraction. Additionally, the failure to reach statistical significance ( $p < 0.05$ ) might be attributed to the heterogeneity associated with the nature of the periodontal disease (variability in pocket depth, amount of alveolar bone loss and host immune response), in contrast to the findings of other reports about healthy orthodontic subjects. However, all included subjects underwent the same pre-orthodontic periodontal therapy to minimize the deleterious effect of the present periodontal disease.

It is important to note that while changes in osteocalcin levels can provide valuable insights into orthodontic therapy, they should be interpreted in conjunction with other clinical and radiographic findings to make informed treatment decisions.

## Conclusions

Within the limitations of the current study, it could be concluded that Periodontal treatment, prior to orthodontic intervention is mandatory. Adult orthodontic treatment has no serious harmful effects on the periodontium as this study showed a nonsignificant increase in the gingival fluid Osteocalcin concentration level through the 6-month follow-up period, which may be positively correlated with the improvement in periodontal parameters.

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