The Effect of Orthodontic Force on Salivary Levels of Alkaline Phosphatase Enzyme

Suha Ali Abdul Ameer, B.D.S., M.Sc. [1]
Akram Faisal Alhuwaizi, B.D.S., M.Sc., Ph.D. [2]

ABSTRACT

Background: Orthodontic tooth movement is characterized by tissue reactions, which consist of an inflammatory response in periodontal ligament and followed by bone remodeling in the periodontium depending on the forces applied. These processes trigger the secretion of various proteins and enzymes into the saliva.The purpose of this study was to evaluate the activity of alkaline phosphatase (ALP) in saliva during orthodontic tooth movement using different magnitude of continuous orthodontic forces.

Materials and Methods: Thirty orthodontic patients (12 males and 18 females) aged 17-23 years with class II division I malocclusion all requiring bilateral maxillary first premolar extractions were randomly divided into three groups according to the magnitude of the force application (40, 60 and 80gm). A sectional fixed appliance was bonded and designed to give labial force to the maxillary first premolar for three weeks. Unstimulated saliva was collected from the patients before force application, then 1 hour after force application, followed by 1 day, 7 days, 14 days and 21 days.Salivary levels of ALP were measured using spectrophotometer and compared with the baseline level.

Results: The results revealed that ALP enzymelevel increased with increasing magnitude of orthodontic force (from 40 to 80gm). This was statistically insignificant after 1 hour and 1 day of force application, but significant after 7, 14 and 21 days.The ALP level significantly increased from baseline after 7 days of force application and peaked at 21 days for all the three force levels.

Conclusions: The ALP level reflect the biological activity that takes place in the periodontium during orthodontic tooth movement, and therefore they can be used as a diagnostic tool for monitoring of correct orthodontic tooth movement in clinical practice.

Key words: Alkaline phosphatase, orthodontic tooth movement, orthodontic force. (J Bagh Coll Dentistry 2015; 27(4):175-179).

INTRODUCTION

Orthodontic tooth movement constitutes a highly complex process defined as an adaptive biological response to interference in the physiological equilibrium in the dentofacial structures by an externally applied force [1]. The host response to orthodontic force has been described as an aseptically and transitory inflammation that mainly alters the vascularity and blood flow of periodontal ligament, resulting in local synthesis and release of different mediators involved in alveolarbone remodeling[2,3].

Progress of tooth movement can be classified into four stages, that is, activation, resorption, reversal, and restructuring of new bones [4]. An early response to orthodontic force is acute inflammation followed by bone resorption and formation. The resorption and formation ofbone are due to increments of activities of osteoclast and osteoblast cells [5].

In order to monitor orthodontic tooth movement non-invasively in human beings, changes have been examined in the profile and levels of various enzymes, cytokines, growth factors, biomarkers and proteoglycans in gingival crevicular fluid and saliva.

Among those components that change and response to orthodontic force are alkaline phosphatase (ALP), tartrate resistance acid phosphatase (TRAP), lactate dehydrogenase (LDH), and aspartate amino transferase (AST)[6,7].

Application of continuous force produces bone resorption and formation at the pressure area with increased activities of both alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) [8,9].

Although the clinical and radiographic follow-up examination remains the basis for patient's evaluation, analysis of saliva, a fluid that contains local and systemically derived markers, may offer the basis for a phase-specific screening of orthodontic tooth movement [10].

The increase in osteoblastic activity during bone formation will be accompanied by an increased expression of an enzyme called alkaline phosphatase[11]. To investigate the bone remodeling pattern based on ALP activity during an orthodontic treatment, body fluids such as saliva can be used [5].

The identification of salivary biomarkers and its use as a diagnostic tool has many advantages. It is much easier to collect, sufficient quantities can be easily obtained for analysis and no specific laboratory devices are necessary. The collection of saliva is also far less invasive compared to other body fluids such as gingival crevicular fluid and serum [12].

(1) Assistant Lecturer, Department of Dentistry, Al-Rafidain University College.
(2) Professor, Department of Orthodontics, College of Dentistry, University of Baghdad.

MATERIALS AND METHODS

Subject Selection:

Thirty patients (12 males and 18 females) were included in this study; who were attending the postgraduate clinic of the Orthodontic Department in the College of Dentistry, University of Baghdad. All patients had Angle's Class II division 1 malocclusion with no or mild crowding (about 2-3mm).

They all required bilateral extraction of maxillary first premolar teeth as part of their orthodontic treatment.Inclusion criteria included age 17-23 years, good general health with no history of any systemic disease, no use of anti-inflammatory drugs before and during the study, no history of any oral habit, good oral hygiene, good periodontal health (probing depth values not exceeding 3 mm in the whole dentition and no radiographic evidence of periodontal bone loss seen in dental panoramic tomography).

Subjects were examined clinically 2 weeks before appliance placement and underwent a session of accurate ultrasonic scaling and polishing and received oral hygiene instructions. Those patients were randomly divided into 3 groups according to magnitude of force application (40, 60 and 80 gm).

Placement of Orthodontic Appliance:

The length of sectional arch wire used was about 50 mm which consist of two parts: The first part was 0.018 inch round stainless steel arch wire with 35mm length, 30mm horizontal end with included non-traumatic coil, the remaining 5mm vertical apically directed. The second part was 0.021x0.025 inch rectangular stainless steel straight wire with length 15mm, 10mm horizontal and 5mm vertical apically directed. The two vertical ends of both wires were welded by welding device in the lab, and act as stopper in front of molar tube to avoid unwanted movements. The first part (round) was inserted in the premolar bracket while the second part (rectangular) was inserted into the molar tube.

Orthodontic brackets and molar tubes were bonded to enamel surface of right and left maxillary first premolars and first molar respectively, using after acid-etching the enamel of teeth. When the bonding material was completely set, the sectional arch wire was checked inside the patient mouth with the vertical arm in touch with the mesial aspect of the molar tube. The arch wire was marked mesial to the first premolar bracket. Then it was removed and a non-traumatic end was made mesially to the first premolar. After reinsertion, cinch back was made distal to the first molar. The arch wire was bent just mesial to the molar tube in a labial direction so that when ligated to the premolar bracket it will apply labial force on the tooth. This force was measured by a strain gauge. The arch wire was ligated to the premolar bracket by a stainless steel ligature.

Saliva Collection:

The patient was instructed not to eat or drink for at least 1 hour before collection of the sample. The patient was asked to sit in a comfortable position and spit or drool out unstimulated saliva into sterile plane plastic test tube for 10 minutes givingabout 5ml of unstimulated whole saliva and put in a cooling box to stop the growth of bacteria.

The samples were taken from each patient immediately prior to fitting the orthodontic appliance at baseline, then after 1 hour, 1 day, 7 days, 14 days and 21 days after force application to the teeth.

Biochemical Assay:

After collection, the whole saliva was clarified by centrifugation for 20 minutes at 3000 RPM to remove insoluble material by using centrifuge machine The supernatants saliva were collected by pipette into eppendrof tubes and frozen at -20^{o}C until biochemical analysis.The analyses of samples were done in the laboratories of the Poison Center of the Specialized Surgeries Hospital to measure the concentrate of ALP in saliva by Colorimetric method (spectrophoto-metrically) at constant temperature of 37^{o} C, with less than 0.05^{o}C fluctuation.

The test for serum-alkaline phosphatase enzyme was done by the use of commercial kit manufactured by Bio Mérieux Sa/France.

RESULTS

The ALP level under different continuous orthodontic force in the 3 groups at the 6 time intervals from baseline to 21 days is shown in table1.

Effect of Force Magnitude:

From baseline to1 day of force application, the ALP levels varied among the 3 groups with insignificantly (p 0.05) as shown in table 2. While, after 7 to21 days of force application, the level of ALP varied among the groups with highly significant difference ($p \leq 0.01$) in which there was a clear increase of ALP concentration with increased force magnitude (Figure 1).

After 7 days of force application, LSD test showed that there was no significant difference (p 0.05) between groups I and II, while there was a highly significant difference ($p \leq 0.01$) between

groups I and III, and between groups II and III (Table 3). After 14and 21 days of force application, LSD test showed that there was significant differences between groups I and II, groups I and III and between groups II and III (Table 3).

Effect of Time Duration of Force:

In each group, after 1 hour of force application, the ALP level slightly increased when compared to the baseline.While after 1 day of force application, the ALP level slightly decreased. After 7 days the ALP level increased and reached at peaked in 21 days as (Table 1). These differences were highly significant for the 3 groups ($p \leq 0.01$) (Table 4).

For the 3 groups, LSD test showed that the differences between baseline, 1 hour and 1 daywerestatistically insignificant. Whereas the differences between 1 day, 7 days, 14 days and 21 days were statistically significant (Table 5).

Table (1): Descriptive Statistics of the Salivary Enzyme ALP level (IU/L).

Duration	Group I (40gm)		Group II (60gm)		Group III (80gm)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Baseline	15.15	2.34	14.76	2.35	14.24	2.38
1 hr	15.24	2.30	14.81	2.35	14.27	2.33
1 day	14.65	2.23	13.71	2.59	13.50	2.44
7 days	16.86	2.00	17.62	1.89	20.24	1.89
14 days	20.27	0.94	22.45	2.08	29.29	2.09
21 days	22.66	1.28	28.96	3.69	39.24	1.73

Table (2): Difference between the Groups for ALP Levels (IU/L) at the 6 Time Intervals Using ANOVA test.

Duration	F-test	d.f.	P value	Sig.
Baseline	0.37	29	0.692	NS
1 hr	0.44	29	0.649	NS
1 day	0.63	29	0.538	NS
7 days	8.45	29	0.001	HS
14 days	69.32	29	0.000	HS
21 days	115.21	29	0.000	HS

NS: Non-significant (p>0.05)
HS: Highly significant (p≤0.01)

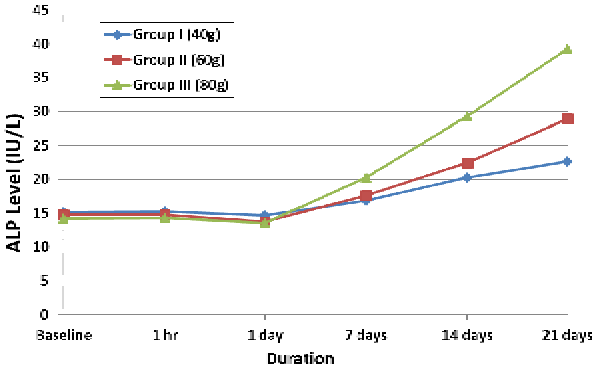

Figure (1): ALP Level IU/L for the Three Groups at the Six Time Intervals.

Table (3): Difference between the Groups for ALP Levels (IU/L) at the 6 Time Intervals Using LSD Test.

Duration	Groups		Mean Difference	S.E.	P-value	Sig.
7 days	I	II	-0.76	0.86	0.385	NS
		III	-3.38	0.86	0.001	HS
	II	III	-2.62	0.86	0.005	HS
14 days	I	II	-2.17	0.80	0.011	S
		III	-9.02	0.80	0.000	HS
	II	III	-6.84	0.80	0.000	HS
21 days	I	II	-6.30	1.10	0.000	HS
		III	-16.58	1.10	0.000	HS
	II	III	-10.28	1.10	0.000	HS

NS: Non-significant (p>0.05), S: Significant (p≤0.05), HS: Highly significant (p≤0.01)

Table (4): Difference between the Six Time Intervals for ALP Levels IU/L for the Three Groups Using ANOVA test.

Groups	F-test	d.f.	p-value	Sig.
Group I (40 mg)	28.85	59	0.000	HS
Group II (60 mg)	53.87	59	0.000	HS
Group III (80 mg)	233.27	59	0.000	HS

HS: Highly significant (p≤0.01)

Table (5): Difference between the ALP Levels (IU/L) between Consecutive Time Intervals Using LSD Test

Duration		Group I		Group II		Group III	
		P value	Sig.	P value	Sig.	P value	Sig.
Baseline	1 hr	0.912	NS	0.961	NS	0.977	NS
1 hr	1 day	0.492	NS	0.339	NS	0.431	NS
1 day	7 days	0.013	S	0.001	HS	0.000	HS
7 days	14 days	0.000	HS	0.000	HS	0.000	HS
14 days	21 days	0.008	HS	0.000	HS	0.000	HS

NS: Non-significant (p>0.05), S: Significant (p≤0.05), HS: Highly significant (p≤0.01)

DISCUSSION

Orthodontics is based on the application of prolonged forces on teeth. Various degrees of force magnitude, frequency, and duration of orthodontic treatment exert a great influence on the surrounding tissue reaction and bone modeling. Interaction between bone formation and resorption during tooth movement results in the release of various biochemical or cellular mediators that can be identified as potential biomarkers [13]. Many studies have investigated possible biomarkers for bone modeling during orthodontic tooth movement such as ALP enzyme which has been associated with bone formation[14-18].

Effect of Force:

In bone modeling process, bone formation occurs between first and second weeks at sites of both tension and pressure. Bone formation has been shown to be represented by the expression of ALP [18].

During bone formation, osteoblasts express ALP [11]. Therefore, the ALP detected in saliva is from the action of osteoblast during bone formation surrounding the teeth. Expression of ALP reflects the biochemical changes that occurs in the supporting tissue after the application of an orthodontic force [19]. Therefore, by monitoring the changes in ALP enzymatic activity, the force application during orthodontic treatment can be customized according to the patient's needs.

In the present study, the salivary ALP level increased with increasing the magnitude of the force and this in agreement with Hong-fei et, al.,[20] who reported that there was direct effect of mechanical strain magnitude on ALP level. However, there was an insignificant decrease in ALP level after 1 day of force application which disagrees with previous studies [14,21]. This could be attributed to the differences in the sampling methods/protocols, processing methodology, sensitivity/specificity of the immunoassays, differences in the type of orthodontic mechanotherapy, force levels, and sample size.

Effect of Time:

In the present study, the level of ALP in the 3 groups significantly increased after 7 to 21 days of orthodontic force application. This may be due to that after orthodontic force application, both bone formation and resorption occur in respective tension or compression sites in order to maintain the integrity of the alveolar bone that holds the dentition. This can be supported by the findings of other studies [11,22] who exhibited that bone formation could be represented by the expression of ALP.Furthermore, during orthodontic force application, there will be destruction of alveolar bone osteoblasts and fibroblasts and their cell membrane will be ruptured releasing their intracellular contents outside. Therefore, ALP will be released into gingival crevicular fluid and saliva and the level of ALP will increase in saliva. This was confirmed by the previous [23,24].

The level of ALP, in this study, significantly increased after 7, 14 and 21 days of orthodontic force application in all 3 groups.This agrees with several authors [14,15,22,25-27]who reported that ALP increased at 1,2 and 3 weeks after force application.

Clinical Significance:

The bony turnover, specifically the bone formation, can be monitored through the expression of ALP level in saliva during orthodontic treatment. Low forces and small tooth movements during treatment may contribute to the low levels of ALP, while high orthodontic forces produce faster tooth movements, as indicated by the significant increases in salivary level ALP activity at 7^{th}, 14^{th} and 21^{st} days during the treatment.

Altogether, these salivary finding might be a reflection of the actual enzymatic profile of gingival crevicular fluid and consequently of the biologic activity within the periodontal environment during orthodontic tooth movement.

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