

Estimation of alkaline phosphatase in the gingival crevicular fluid during orthodontic tooth movement in premolar extraction cases to predict therapeutic progression

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Abstract

Objectives: The objective was to estimate the level of alkaline phosphatase (ALP) in gingival crevicular fluid (GCF) during en-masse retraction stage of orthodontic tooth movement. **Materials and Methods:** 10 patients in the age group of 15-20 years participated in this study. GCF was sampled from the distal surface of the canine and mesial surface of the second premolar on day 0, 1, 7, 14, 21, and 28 postorthodontic treatment. **Results:** A marked fall in the level of ALP was evident following force application. A progressive decreasing trend in ALP activity on both distal aspect of canine and mesial aspect of the second premolar was observed. The fall in ALP was more on distal aspect canine when compared to the mesial aspect of the second premolar. **Conclusions:** Measure of ALP activity in GCF could be an indicator of the biochemical and cellular alterations in bone turnover and hence rate the amount of tooth movement following orthodontic force application.

Key words: Alkaline phosphatase, gingival crevicular fluid, orthodontic tooth movement, osteoclasts

INTRODUCTION

Continued pressure on teeth leading to remodeling of the alveolar bone is the basic principle behind orthodontic tooth movement (OTM). A constant balance between bone formation (on the tension site) and resorption (on the pressure site) facilitates positive remodeling.^[1] However, the biological response of bone turnover may vary between individuals. Gingival crevicular fluid (GCF) is an osmotically mediated inflammatory exudate present

in the gingival sulcus.^[2] Orthodontic forces cause acute inflammatory reaction and vascular changes in the periodontium leading to alterations in the composition of GCF.^[3] Hence, analysis of GCF samples can provide valuable information about periodontal inflammatory/remodeling process as a consequence to OTM. Moreover by monitoring the bone turnover of an individual based on GCF analysis, the amount of force application can be optimized. Several potential biomarkers such as prostaglandins,^[4] Osteoprotegerin,^[5] tumor necrosis factor- α ,^[6] and interleukin-8^[7] are reported in GCF during OTM. While histochemical studies have suggested alkaline phosphatase (ALP) as an important substance appearing during OTM, and indeed bone formation is associated with higher ALP activity.^[2-9] Hence, in the present study, we estimated ALP levels in the GCF as a means to assess progression following orthodontic treatment (en masse retraction stage) and modify the amount of force required based on the levels of ALP as marker of bone turnover.

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MATERIALS AND METHODS

The study involved 10 patients (5 males and 5 females) aged 15-20 years, with no systemic illness and medication history. All the patients had moderate crowding problem and required fixed appliance therapy, which involved the extraction of maxillary first premolar teeth. Prior to commencement of this study, scaling and root planning were performed, and oral hygiene instructions were given to all patients. An informed consent was obtained from each patient before the treatment. Patients were instructed not to use any anti-inflammatory drugs or any mouthwash containing chlorhexidine during the period of the study.^[10]

Periodontal health of the patients was recorded using the following criteria:

1. Full mouth plaque score <20%,
2. Full mouth bleeding score <20%,
3. Periodontal pockets <4 mm,
4. No radiographic bone loss seen in orthopantomogram.

Preadjusted edgewise appliance with 0.018 slot was bonded to the upper and lower teeth. After completion of leveling and aligning, retraction was done on 0.017 × 0.025 stainless steel archwire and elastomeric chain, with a retraction force of about 150 g on each side. The force was measured using digital force gauge.

Gingival crevicular fluid sampling and processing

The teeth to be tested were cleaned with cotton roll and dried using gentle air steam before sample collection. GCF samples were collected from the distal surface of the canine and mesial surface of the second premolar at each time interval from the first quadrant [Figure 1]. GCF was sampled at day 0 (leveling and was recorded as baseline), 1, 7, 14, 21, and 28 days.



Figure 1: Gingival crevicular fluid sample collection

Precut paper filter strips (Whatmann no 1) with a size of 2 mm × 8 mm were used.^[11] The strips were placed in Eppendorf tube and weighed before and after sample collection using digital electronic balance. The strips were inserted into the gingival crevice with 5 s intervals, they were inserted until mild resistance was felt, and kept in the same position for 60 s to increase the volume of GCF collected.^[12] Following GCF collection, the filter strips were immediately placed back in the tube and weighed. The samples were collected in the morning for the entire group of patients and given oral hygiene motivation at each appointment. 50 µl of phosphate buffered saline was added to each sample and mixed with cyclomixer. Centrifugal elution technique was employed to recover the GCF samples from the paper strips. The paper strips from the individual sites were placed in plasto craft and centrifuged twice at 3000 rpm for 2 min. These samples were stored at -80°C until the next procedure is carried out, ALP activity was determined using para nitrophenyl phosphate (PNPP) kinetic method.

Alkaline phosphatase kinetic assay kit

Alkaline phosphatase catalyzes the hydrolysis of PNPP to paranitrophenol and phosphate. Paranitrophenol is yellow in color, as the reaction progresses, the rate of absorbance increases, which is proportional to the activity of ALP sample. This reaction is performed in alkaline medium in the presence of magnesium ions. Reagents used were 2-amino-2-methylpropanol buffer and substrate (PNPP). The change in absorbance was measured at a wavelength of 405 nm as per the instructions in the kit.

Statistical analysis

Data were analyzed using Statistical Package for Social Science Research (SPSS) software package (SPSS Inc., Version 16.0, Chicago). The descriptive statistics, including the mean, standard deviation, and the probability values were calculated for the groups tested. The statistical data were analyzed with ANOVA test and Student's *t*-test. Statistical significance level was established at $P < 0.05$.

RESULTS

Alkaline phosphatase activity changed significantly at various time points following orthodontic force application [Table 1]. The results of ALP activity at 0, 1, 7, 14, 21, 28 days, respectively, point out to a decreasing trend on both distal aspect of canine and mesial aspect of second premolar [Table 1]. The fall in ALP activity was more on the distal aspect of canine when compared to the mesial aspect of the second premolar [Tables 2 to 7]. Thus, indicating a higher osteoclastic activity on the distal aspect of canine compared to the mesial aspect of the second premolar.

Table 1: Comparison between distal aspect of canine and mesial aspect of the second premolar on the basis of different time intervals

Time intervals	n	Mean	SD	F	Significant
Distal aspect of canine					
0	10	50.1000	9.30293	13.531	0.000
1 st day	10	43.9000	7.23341		
7 th day	10	36.8000	8.61265		
14 th day	10	30.7000	9.26223		
21 th day	10	24.4000	11.21705		
28 th day	10	22.2000	8.12130		
Total	60	34.68333	12.44359		
Mesial aspect of second premolar					
0	10	62.9000	13.27027	14.112	0.000
1 st day	10	49.8000	9.67011		
7 th day	10	44.7000	10.11105		
14 th day	10	38.0000	8.35331		
21 th day	10	33.8000	8.43010		
28 th day	10	31.7000	8.15203		
Total	60	43.4833	14.27513		

SD: Standard deviation

Table 2: Comparison between distal aspect of canine and mesial aspect of the second premolar on the initial day (before activation)

Before activation	n	Mean	SD	t	P
Distal aspect of canine	10	50.1000	9.30293	3.941	0.003 (significant)
Mesial aspect of second premolar	10	62.9000	13.27027		

SD: Standard deviation

Table 3: Comparison between distal aspect of canine and mesial aspect of the second premolar on the 1st day (after activation)

After activation	n	Mean	SD	t	P
Distal aspect of canine	10	43.9000	7.23341	2.860	0.019 (significant)
Mesial aspect of second premolar	10	49.8000	9.67011		

SD: Standard deviation

Table 4: Comparison between distal aspect of canine and mesial aspect of the second premolar on the 7th day (after activation)

After activation	n	Mean	SD	t	P
Distal aspect of canine	10	36.8000	8.61265	4.292	0.002 (significant)
Mesial aspect of second premolar	10	44.7000	10.11105		

SD: Standard deviation

DISCUSSION

Our study suggests that ALP is involved in the reaction of bone toward the applied orthodontic forces, an

effect triggered by activity of osteoblastic cells leading to secretion of ALP during bone formation.^[13] Hence, by monitoring the ALP activity, we can check the time course of bone turnover following the orthodontic procedure. Previous studies have indicated that the force exertion by elastomeric chain aids the anterior teeth to move in the distal direction; this movement was a combination of bodily and tipping movement.^[14] As a result, there is a reduction in pure tension and the compression area surrounding the teeth. Hence, concurrent bone formation as well as resorption is observed around the orthodontically moved teeth. Although ALP involvement in bone mineralization is well-known, a few studies have implicated ALP in the synthesis and laying down of the organic matrix only.^[15-17] Interestingly, the activity of ALP is much higher in the periodontal ligament than in other connective tissue.^[18] The ALP activity decreased with the rise in the compressive force. Thus, the fall in activity could probably be due to orthodontic forces being heavier than the physiological range. The pattern of ALP during en-masse retraction stage illustrates the biochemical alterations that take place in the bone enveloping the distal aspect of canine and the mesial aspect of the second premolar. The differential pattern of bone resorption and formation when force was applied to the teeth at the same site is previously reported.^[19] It is assumed that during OTM, there is a reduction in bone formation whereas bone resorption increases, which may be a consequence to force application. Interestingly, the decrease in bone formation and increase in bone resorption in an orthodontically moved tooth around the tooth surface can be estimated by measuring the activity levels of ALP. Indeed few previous studies have iterated this trend.^[20-22]

The biochemical changes in the enveloping bone around canine tooth are reflected in the pattern of ALP during canine retraction stage. Thus indicating that the formation of bone occurs at the tension sites as well as at the pressure site.^[14]

Bone formation occurred more on the mesial surface than on the distal surface, which facilitates preservation of the original tooth's socket morphology.^[12,23] Nevertheless, the amount of bone formation at a specific time correlates with the amount of ALP activity expression in the GCF. Our study supports this concept and emphasizes the utility of estimating ALP levels in GCF to monitor progress of periodontal therapies.

The study has certain limitations, the sample size and monitoring time used are very minimal, which warrant the need for confirming the findings of our study using larger sample size and longer follow-up.

Table 5: Comparison between distal aspect of canine and mesial aspect of the second premolar on the 14th day (after activation)

After activation	n	Mean	SD	t	P
Distal aspect of canine	10	30.7000	9.26223	2.926	0.017 (significant)
Mesial aspect of second premolar	10	38.0000	8.35331		

SD: Standard deviation

Table 6: Comparison between distal aspect of canine and mesial aspect of second premolar on the 21st day (after activation)

After activation	n	Mean	SD	t	P
Distal aspect of canine	10	24.4000	11.21705	5.345	0.000 (significant)
Mesial aspect of second premolar	10	33.8000	8.43010		

SD: Standard deviation

Table 7: Comparison between distal aspect of canine and mesial aspect of the second premolar on the 28th day (after activation)

After activation	n	Mean	SD	t	P
Distal aspect of canine	10	22.2000	8.12130	5.115	0.001 (significant)
Mesial aspect of second premolar	10	31.7000	8.15203		

SD: Standard deviation

CONCLUSION

Biochemical analysis of GCF can provide valuable information on the underlying changes in the periodontium. Estimation of ALP may possibly be an indicator of changes occurring in bone and therefore rate the amount of tooth movement, following force application. A significant variation in the ALP activity in GCF on the side undergoing retraction was observed with a marked fall in the level of ALP beyond 150 g of force application.

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