Bone Turnover Changes During Human Orthodontic Tooth Movement - An assessment of Phosphatases Activity in Gingival Crevicular Fluid

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Abstract

Objective: The purpose of this longitudinal study was to ascertain whether acid and alkaline phosphatase activities in gingival crevicular fluid were indicative of bone turnover changes that occurred during one orthodontic cycle of canine retraction in human subjects.

Materials and Methods: Seven patients (two males and five females); mean age of 23 years were selected and strapped up with pre-adjusted edgewise appliance (MBT 0.022 x 0.028-inch slot). Leveling and aligning was completed prior to distalization of the maxillary canines. The right side maxillary canine was the distalized canine (DC) and hence the experimental tooth whereas the contralateral canine (CC) was the control tooth. From the mesial and distal tooth sites of DC and CC the gingival crevicular fluid (GCF) was collected immediately before canine retraction, an hour after and subsequently weekly for a period of 4 weeks and assayed for phosphatase activities. The results were expressed as total acid and alkaline phosphatases (ACP and ALP) activities determined spectrophotometrically at 30^0C at 405 nm.

Results: Increased ACP activity was observed in the distal site of distalized canine with a significant peak on the 21st day whereas increased ALP activity was observed in the mesial site of distalized canine with significant peaks on the 14th and 28th day.

Conclusion: The alternating peaks of acid and alkaline phosphatase activity in GCF was indicative of alveolar bone turnover changes that occurred during canine retraction indicating that orthodontic tooth movement could be biologically monitored to facilitate patient compliance and optimal force delivery.

Keywords: Acid Phosphatase; Alkaline Phosphatase; Gingival Crevicular Fluid; Orthodontic Tooth Movement

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Introduction

Remodeling characterized by bone resorption on compression side and bone deposition on tension side is a balanced process characteristic of bone turnover occurring during orthodontic tooth movement [1,2].

Orthodontic forces produce distortion of periodontal ligament extracellular matrix resulting in changes in cytoskeletal configuration which leads to synthesis of inflammatory mediators, tissue degrading enzymes, acids, and extracellular matrix components that induce cellular proliferation and differentiation leading to tissue remodeling. Gingival crevicular fluid (GCF) is an osmotically mediated inflammatory exudate and it increases in volume due to inflammation and since orthodontic tooth movement is an inflammatory event, the presence of the above mentioned constituents in GCF would be indicative of changes occurring in the periodontal tissues [3]. Elevations in acid phosphatase levels is associated with bone resorption [4,5] and the same in alkaline phosphatase levels accompanies bone formation (6). Detection of inflammatory mediators like prostaglandins, interleukin-1 beta, interleukin 6, tumour necrosis factor alpha, beta 2 microglobulin, and epidermal growth factor in GCF is indicative of changes occurring deep inside the periodontal tissues [7-9].

Increased levels of chondroitin sulphate was observed in the site of the side to which the teeth were moved orthodontically [10]. Griffiths., et al. [11] reported an increase in the levels of osteocalcin and piridinium cross-links of bone collagen in the GCF in orthodontically treated teeth. It has also been opined that the glycosoaminoglycans in the GCF reflected changes that occurred in the deeper periodontal tissues during orthodontic tooth movement [12]. Increases in lactic and citric acid during orthodontic tooth movement too have been reported [13]. In a longitudinal study, Insoft., et al. [14] concluded that acid and alkaline phosphatase activities can be successfully measured in GCF during orthodontic treatment and that these changes were a reflection of alveolar bone remodeling. Animal studies in rats too suggested that phosphatase activities reflected bone turnover in orthodontically treated tissues [15]. The purpose of this study was to analyse the GCF for acid phosphatase (ACP) and alkaline phosphatase (ALP) activity that occurred during one orthodontic cycle of canine retraction and to conclude whether these phosphatase activities could be used to monitor tissue responses during orthodontic tooth movement.

Materials and Methods

Seven orthodontic patients, 2 males and 7 females, (age range, 14 - 27 years, mean 23 years) were included in the study. The inclusion criteria included a full-mouth plaque score and bleeding score less than or equal to 20% with probing depth values not exceeding 3mm in whole dentition in patients with good periodontal health with no systemic illness. The use of nonsteroidal anti-inflammatory drugs during the study period was strictly prohibited. Informed consent was obtained from the patients and the protocol was viewed and approved by the Ethical Committee of Bharath University, Chennai, India. Fixed appliance therapy (pre adjusted edgewise appliance (MBT, 3M-Unitek; Monrovia, California), 0.022 x 0.028 – inch slot with transpalatal anchorage was strapped up and initial alignment and leveling was completed prior to canine distalization.

In each subject, the maxillary canine on the right side was the distalized canine (DC) whereas the contralateral canine was the control canine (CC). The initial leveling and alignment was accomplished with 0.014-inch and 0.016-inch nickel titanium archwires followed by 0.016 x 0.022-inch stainless steel archwire. 0.017 x 0.025 stainless steel archwire was then placed and prior to commencement of canine distalization the maxillary anterior teeth were passively laced with 0.010-inch stainless steel ligature wire and kept in situ for a month. Subsequently, the ligature wire was then tied passively from CC to the contralateral maxillary lateral incisor and the maxillary right canine was then distalized using a 9 mm nickel titanium coil spring stretched from canine hook to the hook on the maxillary right first molar tube, delivering a constant force of about 250 gm. Probing depth (PD), presence or absence of dental plaque (PL), and bleeding on probing (BoP) was clinically monitored throughout the experimental period.

Isolation with cotton rolls, removal of any supragingival plaque and drying of the experimental and control tooth surface prior to collection of GCF from the crevicular site was the deemed protocol for each subject. A Hirschmann[R] microcapillary pipette, (Sigma Al-

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drichR), was inserted at the mesial and the distal sites of the DC and CC, and one micro-litre of native GCF was collected for ACP analysis before canine distalization, an hour post canine distalization and weekly thereafter for a period of 4 weeks. One micro-litre of GCF fluid so collected was diluted to 99 µL with Sorensens media containinig 0.05% bovine serum albumin in phosphate-buffered saline pH 7.0 in a plastic cuvette. One substrate tablet was dissolved in 2.2 mL buffer solution to prepare the working reagent solution and one mL of the same was added to 100µL of the GCF sample solution and the ACP activity was assayed with a spectrophotometer at 30^0C at 405 nm. The quantitative Acid Phosphatase kit was from, Coral Clinical Systems, Volmolenheide, Belgium, (DEA), (pNPP Kinetic method) with the following composition: Citrate Buffer 50 mmol/L pH 5.2, α-Naphthyl Phosphate > 3 mM, Fast Red TR > 1 mM and preservatives. The above mentioned protocol was adopted for ALP analysis too and one micro-litre of the collected GCF from the crevicular sites was diluted to 100µL with Sorensens media containinig 0.05% bovine serum albumin in phosphate-buffered saline pH 7.0 in a plastic cuvette. The working reagent solution was prepared by dissolving 1 substrate tablet in 3.2 mL buffer solution and to 20 µL of the GCF sample solution in a plastic cuvette the so prepared one mL working reagent solution was added and the ALP activity was assayed with a spectrophotometer at 30^0C at 405 nm. The Alkaline Phosphatase kit used was from Coral Clinical Systems, Volmolenheide, Belgium (DEA), (pNPP Kinetic method) and the composition of it was: DEA, Buffer 1M pH 10.3, magnesium chloride 0.5 mM and pNPP 10 mM.

ACP at an acidic pH hydrolyses α-Naphthylphosphate to form α-Naphthol and Inorganic Phosphate and the α-Naphthol so formed is coupled with Fast Red TR salt to form a diazo dye complex whose rate of formation is measured as an increase in absorbance at 405 nm which is proportional to the ACP activity in the sample. The absorbance was converted into enzyme activity units released per minute at 30^0C. ALP hydrolyses p-nitrophenyl phosphate to p-nitrophenol and inorganic phosphate. The rate of increase in absorbance at 405 nm was monitored as the p-nitrophenol formed. The absorbance was converted into enzyme activity units (1U = 1 mmol of p-nitrophenol released per minute at 30^0C).

Readings for both ACP and ALP activity were noted immediately after initiation of the reaction (A1), 1 minute later (A2), 2 minutes later (A3) and 3 minutes later (A4). The summation of the changes over the 3 minutes period starting from A1 to A4 [(A2-A1) + (A3-A2) + (A4-A3)], was then calibrated and the change in absorbance was noted and designated as delta A. The mean change in absorbance per minute was calculated (delta A/min).

Total acid phosphatase activity in IU/L was calculated using the formula: Delta A/min x 750 and that for alkaline phosphatase activity in IU/L was calculated using the formula: Delta A/min x 2754.

According to the readings obtained in the spectrophotometer a master chart was prepared for the enzyme activity. The mean level of acid and alkaline phosphatase activity was calculated and the standard deviation of the mean values of the enzyme activity at the mesial and distal sites of the experimental and control canines was determined.

Results

There was no clinically detectable movement in CCs, whereas DCs underwent a distal movement of about 1 mm in the period of study of about 28 days. From the ACP activity values obtained at each site from every individual patient included in the study, the mean ACP levels in the GCF at the mesial and distal sites of the DC and the CC was calculated. Independent samples t-tests (parametric tests) was used for making comparison between control and experimental group. Before activation the mean acid phosphatase level in the mesial site of experimental group was equal to the levels noted in control group and the mean ACP level in the distal site of the experimental group was greater than that in the control group. The ACP activity in the distal site (site of compression) of the experimental group was found to increase from an hour after activation to a significant increase on 7th day with a steady increase weekly with a heightened peak of 6.6 on 21st day with a dip to 5.5 on the 28th day. On the mesial site of the experimental group though there was an increase in ACP levels from 7th day to 28th day it was significantly less than that on the distal site. The ACP levels on the mesial and distal sites of the control group were close to baseline values.

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On the 28th day the acid phosphatase activity in the mesial site of the distalized canine of the experimental group was 1.3 fold higher when compared to the mesial site of the control group and the same in the distal site of the distalized canine was 2.7 fold higher when compared to the distal site of the control group (Table 1a).

Mean acid phosphatase activity	Experimental group				Control group			
	Mesial		Distal		Mesial		Distal	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Before activation	1.821	0.400	2.357	.674	1.821	.400	1.928	.590
1hr	1.928	.590	2.142	1.008	2.035	.365	1.607	.674
7th day	3.107	.517	3.964	.940	2.464	.365	2.464	.566
14th day	3.642	.517	4.607	.801	2.571	.400	3.000	.433
21th day	4.928	.400	6.642	.801	2.785	.940	3.535	.365
28th day	4.071	.400	5.464	.834	2.678	.850	2.678	.400

Table 1a: *Mean acid phosphatase activity.*

From the ALP activity values obtained at each site from every individual patient included in the study, the mean ALP levels in the GCF at the mesial and distal sites of the DC and the CC was calculated. It was inferred that the ALP activity in GCF was found to be different in both mesial and distal sites in experimental as well as in control groups. As far as experimental group was concerned, ALP activity in GCF was found to steadily increase one hour after activation in the mesial site (site of tension) with a steady increase weekly. It rose to the level of 47.6 on the 14th day, dipped to 37.4 on the 21st day and peaked to 50.8 on the 28th day. In the control group, the ALP activity in GCF in the mesial site showed an insignificant increase from 7th day onwards and reached the highest level of 22.8 on the 28th day but was significantly less in comparison to the mesial site in the experimental group. Though ALP activity in GCF in the distal site of experimental group rose slightly weekly it was certainly significantly less than in the mesial site of the experimental group. The ALP activity in GCF in the distal site of control group was found to remain at the same level before activation, and one hour after activation. There was an insignificant increase in levels on 14th, 21st and 28th days, which was significantly less in comparison to the distal site of experimental group.

Before activation the alkaline phosphatase activity in the mesial site of the distalized canine was comparable to that of the mesial site of the control group and the distal site of the experimental group was lesser than that of the control group. On the 28th day the alkaline phosphatase activity in the mesial site of the distalized canine of the experimental group was 27.8 fold higher when compared to the mesial site of the control group and the same in the distal site of the distalized canine was only 12.9 fold higher when compared to the distal site of the control group (Table 1b).

Mean alkaline phosphatase activity	Experimental group				Control group			
	Mesial		Distal		Mesial		Distal	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Before activation	14.950	1.472	12.983	2.619	14.950	2.166	14.556	2.081
1hr	18.491	1.343	15.266	2.121	15.343	2.687	14.950	2.166
7th day	26.359	1.472	20.851	2.166	18.491	1.343	16.130	1.900
14th day	47.604	1.343	30.294	4.206	20.851	4.163	18.884	2.944
21th day	37.375	6.128	27.146	3.346	20.458	3.122	18.491	2.619
28th day	50.752	2.687	32.654	4.882	22.818	2.6195	19.671	2.477

Table 1b: *Mean alkaline phosphatase activity.*

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The paired t test for within group compared the ACP activity in the experimental group, before activation and 28 days after activation in both the mesial and distal sites. The ACP activity before activation and on 28th day in the distal site was statistically significant with a P value of 0.000, and the mean ACP value in the distal site of the experimental group was more than that in the mesial site, as it was the site of compression. The paired t test for within group also compared the ACP activity in the control group, before activation and 28 days after activation in both the mesial and distal sites and the ACP activity before activation and on 28th day in the distal site was less statistically significant when compared to its counterpart in the experimental group. There was a significant increase in acid phosphatase activity after 28 days in both the mesial and distal sites of the experimental group, however, the increase in mean level in the distal site of the experimental group was significant (Table 2a).

Group	Site	Mean	SD	t	df	Sig	95% CI	
							Lower limit	Upper limit
ACP Experimental Group	Before Activation- Mesial	2.250	.612	-9.721	6	0.000	-2.816	-1.683
	28th Day After Activation-Mesial							
	Before Activation- Distal	3.107	.283	-29.000	6	0.000	-3.369	-2.844
	28th Day After Activation- Distal							
ACP Control Group	Before Activation- Mesial	-.857	.674	-3.361	6	.015	-1.481	-.233
	28th Day After Activation- Mesial							
	Before activation-Distal	-.750	.750	-2.646	6	.038	-1.443	-.056
	28th Day After Activation- Distal							

Table 2a: *Paired t-test for within group (ACP).*

The paired t test for within group also compared the ALP activity in the experimental group, before activation and 28 days after activation in both the mesial and distal sites. The ALP activity before activation and on 28th day in the mesial site and distal sites was statistically significant with a P value of 0.000. The paired t test for within group compared the ALP activity in the control group, before activation and 28 days after activation in both the mesial and distal sites and the ALP activity before activation and on 28th day in the mesial and distal sites was statistically significant, indicating an increase in alkaline phosphatase activity after 28 days. The increase in alkaline phosphatase activity after 28 days in both the groups was significant (Table 2b).

Group	Site	Mean	SD	t	df	Sig	95% CI	
							Lower Limit	Upper Limit
ALP Experimental Group	Before Activation Mesial	-35.802	2.754	-34.395	6	.000	-38.34	-33.25
	28th Day After Activation-Mesial							
	Before Activation Distal	-19.671	6.444	-8.076	6	.000	-25.63	-13.71
	28th Day After Activation- Distal							
ALP Control Group	Before Activation Mesial	-7.868	4.031	-5.164	6	.002	-11.59	-4.14
	28th Day After Activation- Mesial							
	Before Activation Distal	-5.114	1.900	-7.120	6	.000	-6.872	-3.356
	28th Day After Activation- Distal							

Table 2b: *Paired t-test for within group (ALP).*

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The t-test compared the 2 groups at 95% Confidence Interval. It showed a significant difference between the two groups with the experimental group showing a significant activity in acid and alkaline phosphatase levels than the control group (Table 3).

Site	Group	Mean	SD	t	df	Sig	95% CI	
							Lower Limit	Upper Limit
ACP activity mesial surface	Exp.	4.071	.400	3.920	12	0.002	.582	2.203
	Control	2.678	.850					
ACP activity distal surface	Exp	5.464	.834	7.961	12	0.000	1.988	3.582
	Control	2.678	.400		12			
ALP activity mesial surface	Exp	50.752	2.68	19.692	12	0.000	24.842	31.024
	Control	22.81	2.619			0.000		
ALP activity distal surface	Exp	32.65	4.882	6.274	12	0.000	8.293	17.672
	Control	19.67	2.477					

Table 3: *t-test for comparison between groups.*

The co-efficient of variance between the two groups associated with tooth movement based on acid phosphatase activity in mesial site was 21.9% and in distal site was 0.3% and the alkaline phosphatase activity in mesial and distal sites were 6.2% and 2.4% respectively. Therefore the acid and alkaline phosphatase activity in the mesial site of the experimental group was higher than the control group after 28 days of activation (Table 4).

Group	Cv (%)
ACP activity on mesial surface	21.9
ACP activity on distal surface	0.3
ALP activity on mesial surface	6.2
ALP activity on distal surface	2.4

Table 4: *Coefficient of variance between two groups.*

Discussion

Acid phosphatase (ACP) and alkaline phosphatase (ALP) belong to the hydrolase class of enzymes and while the former is found in high concentrations in the liver, RBC's, and prostate the latter is in liver, biliary tract epithelium and bone. Elevations in ACP activities is associated with bone resorption [5]. ALP is an essential enzyme for bone deposition [16] and thus a reliable marker of osteoblastic activity [17,18] and acts by hydrolyzing nonorganic pyrophosphate, a potent inhibitor of the mineralization process [19]. Its level and activity varies in physiological events such as bone development, and pathological events such as rickets, Paget's disease, osteomalacia, hyperparathyroidism, liver disease, and inflammatory bowel disease.

It has been reported that bone remodeling with deposition in tension sites and resorption in pressure sites is a characteristic feature of orthodontic tooth movement with confirmatory evidence gleaned from animal studies too [20,21]. However, remodeling is a complex process and it has been histologically observed that bone resorption and deposition takes place in both the compression and tension sites of the alveolar bone [22]. GCF constituents serve as diagnostic markers of active tissue destruction occurring deeper in periodontal tissues [23,24]. Alkaline phosphatase, aspartate aminotransferase, β-glucoronidase, immunoglobulin G4 and prostaglandins are the only components among more than 40 components studied that associate with clinical measures of periodontal disease progression at spe-

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cific sites [25-28]. However, few studies have reported their role in orthodontic tooth movement (OTM) bone remodeling. The subjects recruited for the study, were of good general health to exclude any ambiguity of changes in CGF constituents due to periodontal diseases.

Collection of GCF can be done by various methods. The paramagnetic bead is ideal for capture of tumour necrosis factor directly from the gingival sulcus as it is covered with anti-TNF monoclonal antibodies which forms complexes with the TNF. The beads are then retrieved with a special magnetic harvester.

A prewashed absorbent string inserted into the gingival crevice too can be used for GCF collection, but could involve problems with accurate weighing [29].

Placement of filter paper strips in the gingival crevice is also a widely used method as it is less disruptive to the delicate crevicular epithelium [30,31].

Predetermined volumes of GCF can be collected with micro-capillary tubes too [32,33]. The Hirschmann microcapillary pipette was used to collect 1 µL of native GCF. It was passed back and forth in the gingival crevice for 10 - 15 minute predetermined short length of time with extreme caution exercised to not disrupt the delicate crevicular epithelium nor to prevent contamination with blood or serum as any increase in GCF volume can dilute its contents. Gingival crevicular fluid ACP and ALP activity is the total activity per sample and small errors in volume determination can lead to large errors in estimates of fluid concentrations [34].

The DCs underwent a distal movement of about 1mm in the period of study of about 28 days, whereas there was no clinically detectable movement in CCs. The ACP activity in the distal site (site of compression) of the experimental group was found to increase steadily from an hour after activation to a significant increase on 7th day with a heightened peak on 21st day with a dip on the 28th day. The increase in ACP levels in the mesial site of the experimental group from 7th day to 28th day was significantly less than that on the distal site (Figure 1 and 2). On the 28th day the ACP activity in the distal site of the distalized canine was 2.7 fold higher when compared to the distal site of the control group, whereas the mesial site of the distalized canine of the experimental group was just 1.3 fold higher indicating the fact that ACP activity was higher in the distal site (site of compression).
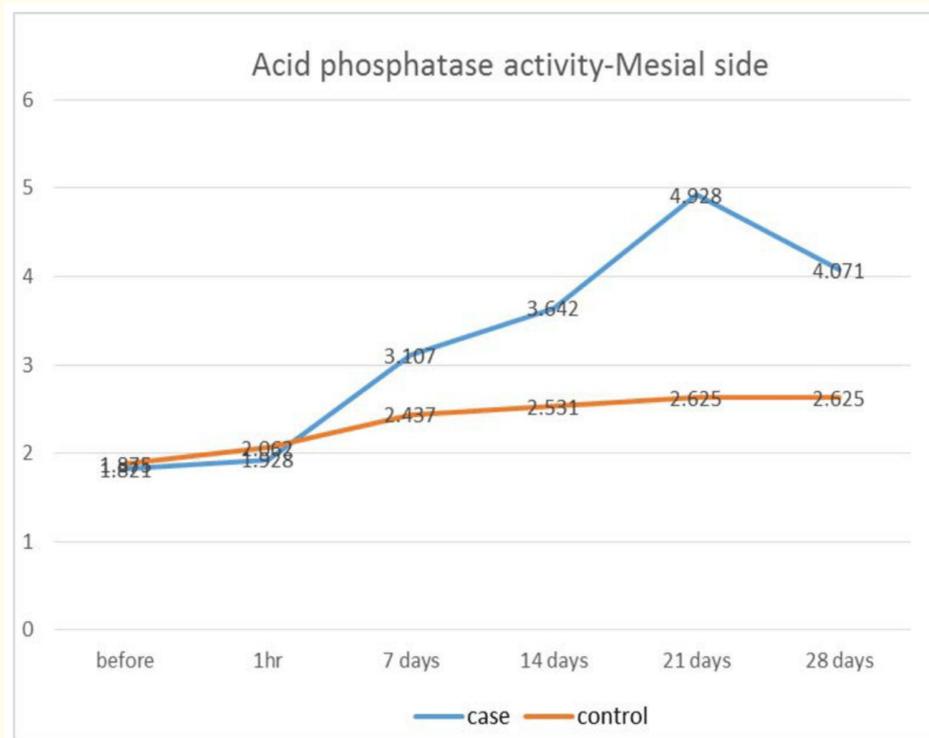

Figure 1: *ACP activity in GCF in mesial sites of experimental and control maxillary canines.*

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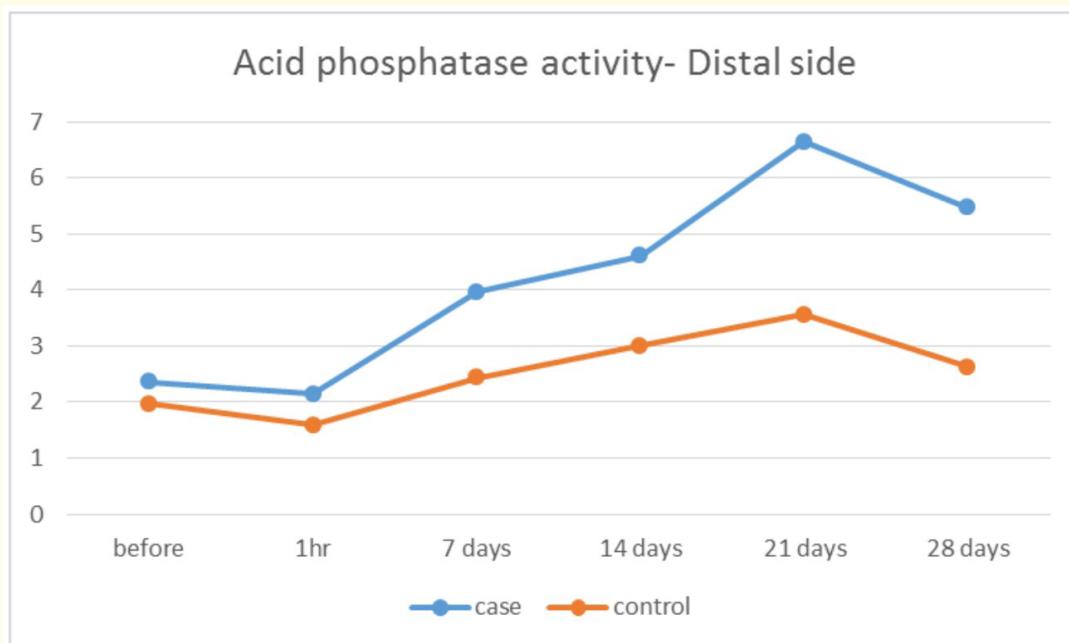
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Figure 2: *ACP activity in GCF in distal sites of experimental and control maxillary canines.*

In the experimental group too the ALP activity in GCF was found to steadily increase an hour after activation in the mesial site (site of tension) with an increase on the 14th day to a heightened peak on the 28th day whereas in the control group, the ALP activity in GCF in the mesial site showed an insignificant increase from 7th day to 28th day (Figure 3 and 4). On the 28th day the alkaline phosphatase activity in the mesial site of the distalized canine of the experimental group was 27.8 fold higher when compared to the mesial site of the control group and the same in the distal site of the distalized canine was only 12.9 fold higher when compared to the distal site of the control group.
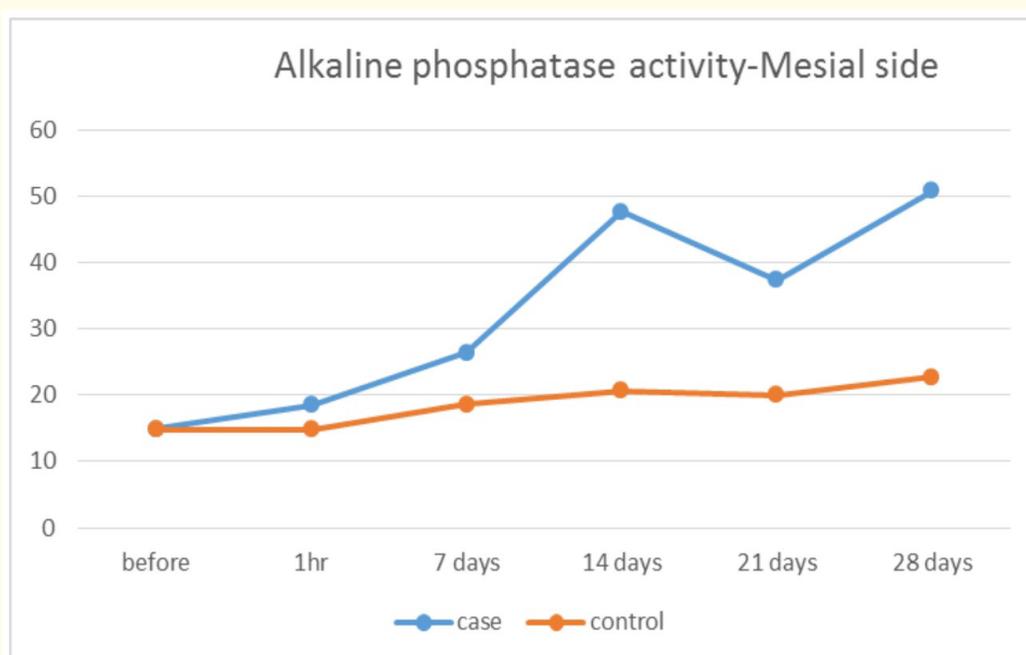

Figure 3: *ALP activity in GCF in mesial sites of experimental and control maxillary canines.*

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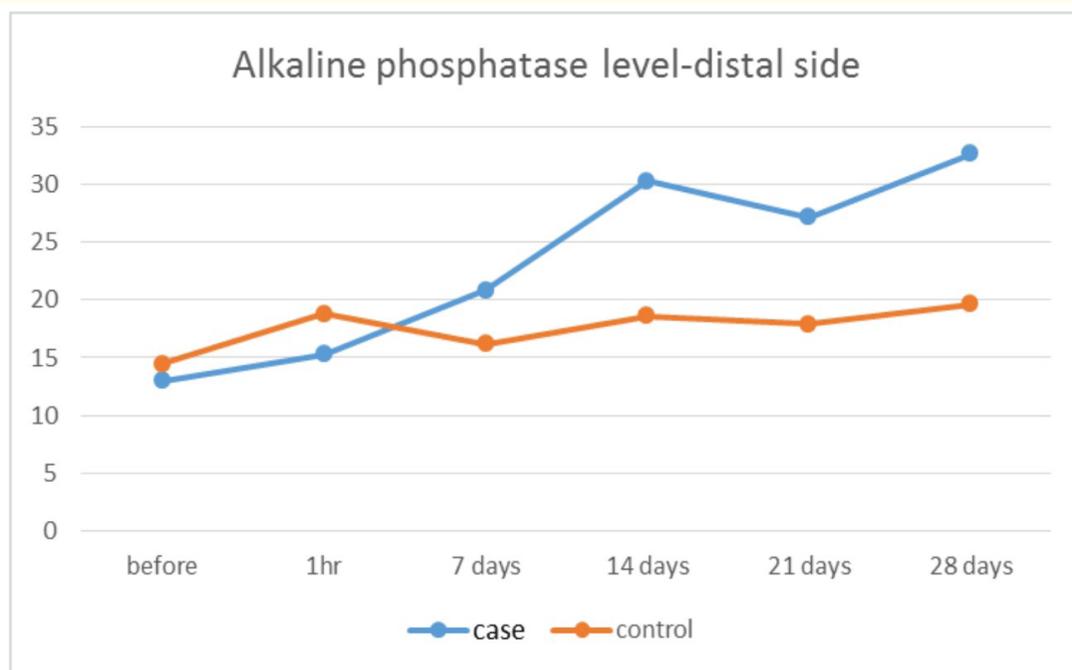
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Figure 4: ALP activity in GCF in distal sites of experimental and control maxillary canines.

There was a significant increase in acid phosphatase activity after 28 days in both the mesial and distal sites of the experimental group, however, the increase in mean level in the distal site of the experimental group (site of compression) was significant. The ALP activity before activation and on 28th day in the mesial and distal sites was statistically significant, indicating an increase in alkaline phosphatase activity after 28 days. The increase in alkaline phosphatase activity after 28 days in both the groups was significant.

The experimental group showed a significant activity in acid and alkaline phosphatase levels than the control group and though acid and alkaline phosphatase activity was found in both the mesial and distal sites, the same in the mesial site of the experimental group was higher than the control group. However, on the 28th day that is after a period of one orthodontic cycle it was found that there was a significant peak in ACP and ALP activity on the distal site and mesial site respectively of the experimental group indicating optimal remodeling of bone around the distalized canine. The data showed period of peak tooth movement with acid phosphatase activity and little tooth movement with elevations in alkaline phosphatase activity. These observations suggest that GCF phosphatase activities may reflect biologic processes associated with orthodontic tooth movement as had been widely reported in literature [35]. It may be argued that phosphatase activities in the GCF can be influenced with bone turnover that may occur during growth and in metabolic diseases [36]. Hence, we had carefully chosen healthy adult patients with no metabolic diseases and who were past active growth, so that the phosphatase changes that were observed in the GCF were truly indicative of bone turnover changes occurring during OTM. Data has also shown that alkaline phosphatase activity increases with inflammation [37] and as OTM is an inflammatory phenomenon its detection in GCF is also indicative of bone turnover changes. Gingival inflammation too can cause increased ALP activity [38]. As the plaque score and the bleeding on probing score was less than 20%, it was deduced that the increase in ALP activity was due to a mechanically induced inflammation and not a bacterially induced one. Histological studies too have reported the association of increased ACP activity in GCF and rapid OTM [39]. Researchers observed a high ALP activity in tension sites and decreased enzymatic activity in pressure sites in rats [40]. Takemoto.,

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et al. [41] has opined that ALP is more important in monitoring OTM as it increases greatly over control, while the case is not so for ACP where it does not differ considerably from control. Investigators have also reported a time dependent increase in ACP and ALP levels at both pressure and tension sites with an early increase in ACP followed by reversal and then a later peak in ALP, as had been observed in the study too. The oscillating nature and inverse relationship of ACP and ALP activity suggest that alveolar bone remodeling dynamics in human OTM is characterized by periods of activation, resorption, reversal and formation and that these cycles which last for 4 months are also asynchronous in nature [42,43].

The drawbacks of the study could be due to any of several methodologic variables, like sampling device (filter paper strips could have been used instead of microcapillary tubes), method used for phosphatase analysis and data handling. Despite ACP and ALP being good markers for metabolic bone activity neither is exclusively found in bone, and since tartrate resistant acid phosphatase and levimasole inhibited ALP activities are more specific to bone cells, assaying of the same could be more predictive of bone turnover activity. Nevertheless, it has been reported that ALP and ACP activity increases as the periodontal ligament is in tension and in compression, and the estimation and analyses of these phosphatases in the GCF could surely enable the orthodontist to monitor and predict the biologic processes occurring during OTM. Hence, phosphatase analyses is an invaluable diagnostic tool in clinical practice to aid the orthodontist to deliver optimal orthodontic forces and based on individual tissue responses manage the appliance effectively.

Conclusions

1. Increased ACP activity was observed on the distal site of the distalized canine with a significant peak on 21st day and increased ALP activity was observed with significant peaks on the 14th and 28th day in the mesial site of the distalized canine.

2. On the 28th day the acid phosphatase activity in the distal site of the distalized canine was 2.7 fold higher when compared to the distal site of the control group, indicating the fact that ACP activity was higher in the distal site (site of compression).

3. On the 28th day the alkaline phosphatase activity in the mesial site of the distalized canine (site of tension) of the experimental group was 27.8 fold higher when compared to the mesial site of the control group, indicating the fact that ALP activity was higher in the mesial site (site of tension).

4. Acid and alkaline phosphatase activity in the mesial site of the experimental group was higher than in the control group.

5. ACP and ALP activity on the control side showed values near to baseline scores on both the mesial and distal sites.

6. Significant variations in acid and alkaline phosphatase levels in GCF observed in both the mesial and distal sites of the experimental group were indicative of alveolar bone remodeling and an analysis of the same would aid the orthodontist to biologically monitor and predict OTM.

Support

Nil.

Conflicts of Interest

Nil.

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