

Gingival Crevicular Fluid Flow Rate and Alkaline Phosphatase Level as Potential Marker of Active Tooth Movement

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Abstract

Background: Gingival Crevicular Fluid (GCF) changes occur during orthodontic tooth movement and this could serve as a potential indicator to the response to active treatment.

Aim: The objective of the study is to assess the changes in the GCF volume and the levels of Alkaline Phosphatase (ALP) during early phase of tooth movement.

Methods: 20 patients requiring all first premolar extractions were selected and treated with conventional straight wire mechanotherapy. Canine retraction was done using Nitinol closed coil springs. Maxillary canine on one side acted as experimental site while the contralateral canine acted as control. GCF was collected from around the canines before initiation of retraction, 1 hour after initiating canine retraction, 1 day, 7 days, 14 days and 21 days. GCF volume and the ALP levels were estimated and compared with the control side.

Results: The results showed statistically significant changes in the GCF volume and ALP levels on the 7th, 14th and 21st days at the experimental sides. The peak in the activity occurred on the 14th day of initiation of retraction. The GCF volume and ALP levels did not show any significant variations at the control sites where no retraction was done.

Conclusions: It can be concluded that GCF volume and ALP levels may serve as an indicator to assess tooth movement dynamics in orthodontic therapy. Based on the available data and further studies, ALP levels in GCF may aid in developing a reliable non-invasive chair side test for assessing the prognosis and progress of orthodontic therapy.

Key words: Gingival crevicular fluid, Orthodontic tooth movement, Canine retraction, Alkaline phosphatase

Introduction

Tooth movement by orthodontic treatment is characterized by remodeling changes in dental and periodontal tissues, including dental pulp, periodontal ligament, alveolar bone, and gingiva. These force-induced strains alter the periodontal ligaments' vascularity and blood flow, resulting in local synthesis and release of various key molecules, such as neurotransmitters, cytokines, growth factors, colony-stimulating factors, and arachidonic acid metabolites. These molecules can evoke many cellular responses by various cell types in and around teeth, providing a favorable microenvironment for bone deposition or resorption [1,2]. The biomechanical principles of tooth movement during orthodontic treatment have been extensively described [3,4]. These are supported by several studies that have evaluated periodontal changes incident to orthodontic tooth movement [5-8].

The early phase of orthodontic tooth movement always involves an acute inflammatory response, characterized by periodontal vasodilatation and migration of leukocytes out of the capillaries. A reflection of these phenomena can be found in the gingival crevicular fluid of moving teeth, where significant elevations in the concentrations of inflammatory mediators, such as cytokines and prostaglandins was reported [9]. Gingival crevicular fluid arises at the gingival margin and can variously be described as a transudate or an exudate. Its rate of flow is related to the degree of gingival inflammation, and a rate of 0.05 to 0.20 μL per minute was reported in cases of apparent minimal inflammation. The total fluid flow

is between 0.5 and 2.4 μL per day [10]. Several studies have focused on the composition of gingival crevicular fluid and the changes that occur during orthodontic tooth movement [11,12]. Gingival crevicular fluid component analysis is a noninvasive method for studying the cellular response of the underlying periodontal ligament during orthodontic treatment [13].

Alkaline Phosphatase is a glycoprotein and membrane bound enzyme. It hydrolyzes monophosphate ester bonds at alkaline pH, increasing local concentrations of phosphate ions. In the periodontium, alkaline phosphatase is a very important enzyme as it is part of the normal turnover of periodontal ligament, cementum, and bone homeostasis. It is produced by many cells, including fibroblasts, osteoblasts and osteoclasts, but the main source of alkaline phosphatase in gingival crevicular fluid is neutrophils [14].

Bone turnover during orthodontic tooth movement has been described as a continual and balanced process characterized by bone deposition at sites of tension and bone resorption on the pressure sites [15,16]. Bone-forming cells have been shown to have alkaline phosphatase activity, and changes in this enzyme in serum and bone have been used as markers for bone metabolism in several diseases [17]. Alkaline Phosphatase Levels (ALP) in gingival crevicular fluid is higher than in serum. In serum, the enzyme is associated with systemic bone disease, and its elevation in gingival crevicular fluid could well reflect changes of alveolar bone in localized areas [18,19]. Monitoring acid and alkaline phosphatase activities in

serum and tissues is a common means to assess bone turnover in human subjects.

Takimoto [20] detected acid and alkaline phosphatase histochemically in periodontal tissues after experimental tooth movement in sixty Wistar strain rats. Insoft [21] examined acid and alkaline phosphatase activities in gingival crevicular fluid to learn whether bone turnover dynamics can be monitored in human subjects during orthodontic tooth movement. They observed a peak alkaline phosphatase level between the first and third weeks after orthodontic activation. Studies have also shown an increase in the level of ALP during orthodontic tooth movement by estimating the levels at tension side and the compression side of the tooth [22-24]. Batra [23] showed significant changes in alkaline phosphatase activity on the 7th, 14th and 21st day of canine retraction with a peak activity on the 14th day.

Analysis of gingival crevicular fluid samples may be a good means of examining the ongoing biochemical processes associated with bone turnover during orthodontic tooth movement [5,11,25-27]. This can be used as an effective prognostic indicator in orthodontic therapy. Monitoring the enzymatic activity of ALP during an orthodontic treatment, the application of orthodontic force can be personalized to the patients' biological needs. Additionally, the understanding of the GCF bone turnover markers could solve the issues related to the retention and relapse during orthodontic therapy.

Even though few isolated studies have shown variation in the GCF flow rate and constituents, the longitudinal studies are scarce and more research has to be done to establish the earlier observations. Hence the current study is done to estimate the GCF volume and the alkaline phosphatase activity in subjects undergoing orthodontic treatment. It can be hypothesized that orthodontic tooth movement influences the GCF volume and the ALP levels. The comparison was done with the contralateral side as control in the same individuals.

Materials and Methods

Study population

A sample of 20 subjects (10 males and 10 females) requiring orthodontic treatment was taken for this study with an age range 15 years to 25 years. The protocol of the present study was reviewed and formally approved by the Ethical Committee of the College of Dentistry Research Centre (CDRC). Informed consent was also obtained from each subject, after explaining the nature of the study. The subjects had Angle's Class I malocclusion with bimaxillary dentoalveolar protrusion and proclination with minimal or no crowding and all required the extraction of all four first premolar teeth as part of their orthodontic treatment. Treatment plan constituted of fixed orthodontic therapy with extraction of first bicuspids, followed by individual canine retraction, and maximum anchorage conservation, space closure, finishing and detailing and a fixed lingual retainer.

The subjects selected were free of oral and systemic diseases, had no periodontal pockets, and had not been on a regimen of antibiotic therapy for at least 3 months prior to the commencement of the study. The subjects were willing to strictly adhere to the oral hygiene instructions provided by the

investigator and agreed to follow the oral hygiene program and orthodontic treatment prescribed for them.

Orthodontic treatment

All patients were treated with conventional straight wire (0.022 x 0.028) mechanotherapy (Discovery[®] brackets, Dentaaurum, Ispringen, Germany). The first premolar extractions were done at the start of treatment or at least 3 months before the commencement of canine retraction so that the bone remodeling occurring due to the healing socket would not influence our study. The patients were banded and bonded using 0.022" slot preadjusted edgewise brackets system (Discovery brackets, Dentaaurum Ispringen, Germany). Leveling and alignment was completed by 3 to 4 months from the start of treatment. Following leveling and alignment, the retraction of the canine was initiated on a base wire of 0.019 x 0.025" Stainless Steel preformed of standard arch form (Arch wires[®], 3M Unitek, Monrovia, California, USA). Canine retraction was performed, on one side chosen at random, using Nitinol closed coil spring (9 mm) capable of delivering approximately 125 g of constant force (Nitinol closed coil spring[®], 3M Unitek, Monrovia, California, USA). The side of canine retraction (experimental site) selection was done randomly by a lottery method. The canine on the contralateral side, acted as control.

Periodontal examination

Thorough oral prophylaxis was done two weeks prior to collection of samples. All patients complied with strict oral hygiene instructions to rinse twice daily with 0.5 ounces of 0.2% chlorhexidine gluconate throughout the study period and to brush their teeth at least two times a day with a tooth brush and tooth paste. Periodic recall was done to evaluate the level of oral hygiene. Patients were instructed not to take any medications or drugs including Non-steroidal anti-inflammatory drugs during the study period.

Gingival crevicular fluid collection

Gingival Crevicular Fluid (GCF) samples were collected according to the method of Lamster [28]. The individual crevicular sites were isolated with cotton rolls and gently air dried. Then, six pre-cut methylcellulose filter periopaper strips[®] (Periopaper strips[®], ProFlow Inc., Amityville, NY, USA) were inserted into the crevice at the mesio-labial line angle, mid-labial surface, disto-labial line angle, disto-palatal line angle, mid-palatal surface and mesio-palatal line angle until mild resistance is felt. These were left in place for 60 seconds while maintaining isolation. The six strips were immediately placed in individual sealed plastic tubes (Cryotube[®], 2.0 mL, NUNC, Roskilde, Denmark) and snap frozen at -70°C until further processing was carried out. The GCF was collected from the maxillary canines before canine retraction was initiated, (a) after initiating canine retraction, 1 hour (b), and then after the following 1 day (c), 7 days (d), 14 days (e) and 21 days (f).

Gingival crevicular fluid volume measurement

The gingival crevice fluid (GCF) was measured using Periotron Model 8000[®], (Harco Electronics Ltd., Zografou, Athens Greece). Calibration of the equipment was done each time as per the manufacturer's recommendations. The Periotron[®] is a micro-moisture meter electronic instrument that has been designed to permit greater use of a variety of paper collection strips for gingival crevicular fluid measurements.

Gingival crevicular fluid alkaline phosphatase assay

Alkaline Phosphatase Flex reagent cartridge (ALP Flex[®] reagent cartridge, Dade Behring Inc., Eschborn, Germany), used on the Dimension[®] RXL Clinical Chemistry Analyzer with Integrated multisensor technology with Heterogeneous Module (Dimension[®] RXL Clinical Chemistry with IMT HM, Dade Behring Inc., Eschborn, Germany), is an in vitro diagnostic test intended for the quantitative determination of alkaline phosphatase activity in serum and plasma. The alkaline phosphatase method is based on a procedure described by Bowers and McComb [29] and was again reviewed by Rej [30]. This method responds to all alkaline phosphatase isoenzymes in human serum and hence has been adopted for our study for the quantitative measurements of gingival crevicular fluid alkaline phosphatase [23].

Sample preparation

One microliter of gingival crevicular fluid was diluted to 100 μ l with phosphate-buffered saline (pH 7.0). The samples were centrifuged at 2000 rpm in a refrigerated microcentrifuge for 1 minute (min) to remove the bacterial and cellular debris. Then the samples were stored at -70°C after adding a drop of acetic acid stabilizer.

One microliter of the reagent solution was added to the gingival crevicular fluid sample and the absorbance was measured in a spectrophotometer[®] (spectrophotometer[®], Model 8453, Hewlett Packard, Waldgrohn, Germany) at 405 nm. Readings were recorded immediately after initiation of the reaction (A1), 1 min later (A2), 2 min later (A3) and 3 min later (A4). The change in absorbance was noted by summation of the changes over the 3-min period starting from A1 to A4 [(A2) A1] + (A3) A2 + (A4) A3] and was designated as delta A. Mean change in absorbance per minute was calculated (delta A/min). Total alkaline phosphatase activity was calculated using the formula:

$$U/L = 3300 \times \text{delta}/\text{min}$$

Assay values are reported in international unit per liter (IU/L).

Statistical analysis

All measurements were statistically evaluated using GraphPad Instat version 3.05 (GraphPad Software Inc. San Diego, CA, USA). Mean values and Standard Deviations (SD) were calculated. The method of Kolmogorov and Smirnov was used to confirm that the data were sampled from populations that follow Gaussian distributions. For comparison of data, repeated measurements of one-way Analysis of Variance (ANOVA) were used with a Tukey's post-hoc test. Differences were considered statistically significant at $p < 0.05$.

Results

GCF volume

The gingival crevicular fluid volume is shown in *Table 1* and *Figure 1*. The gingival crevicular fluid volume showed a tendency to increase with time at the site where orthodontic force is applied. The increase in the gingival crevicular fluid volume was found to be more at the experiment side; it increased gradually from 0 hour to 21 days from 0.58 ± 0.11 μ L to 0.75 ± 0.13 μ L reaching maximum level on the 14th and 21st days. The increase in the gingival crevicular fluid volume was found to be statistically significant on the 14th day.

Table 1. Gingival crevicular fluid volume (μ L) at the control and experiment side.

Time Points	Control	Experiment
	Mean \pm SD	Mean \pm SD
Hour 0	0.53 ± 0.10	0.58 ± 0.11
Hour 1	0.53 ± 0.09	0.68 ± 0.10
Day 1	0.53 ± 0.09	0.69 ± 0.11
Day 7	0.53 ± 0.08	0.73 ± 0.11
Day 14	0.56 ± 0.10	$0.75 \pm 0.12^*$
Day 21	0.57 ± 0.10	0.75 ± 0.13

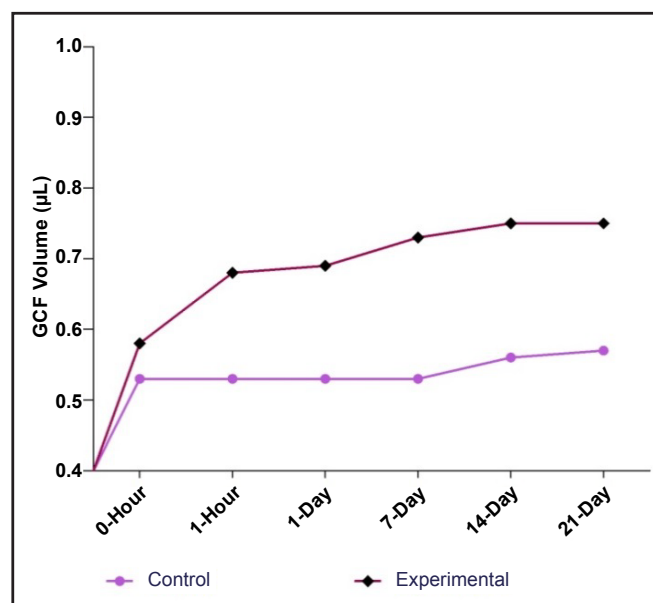


Figure 1. Gingival crevicular fluid volume (μ l) at the control and experiment side.

Using the parametric One Way Analysis of Variance (ANOVA) test for any significant difference within either the control or experiment side at the different points of time, a statistical significant difference was found only in the experiment side.

Gingival crevicular fluid alkaline phosphatase level

The gingival crevicular fluid alkaline phosphatase level in control and experiment sides is shown in *Table 2* and *Figure 2*. The level of alkaline phosphatase in the experiment side increased gradually from 0 hour to 14 days; it was found to be statistically significant on the 14th day with a peak level of 56.75 ± 11.50 IU/L on the 14th day whereas in the control side, there was only minimum variation from 0 hour to 21 days in all the 20 subjects studied and the values were found to be not statistically significant. Using Tukey-Kramer multiple comparison tests, this statistical significant difference was found on 14th day at the experimental side compared to control side ($P < 0.05$).

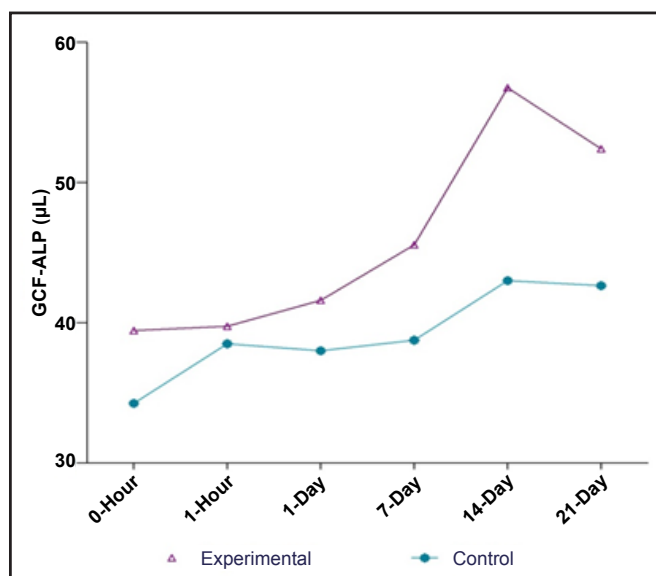
Discussion

Bone remodeling is a dynamic interaction between bone-forming osteoblasts and bone-resorbing osteoclasts. The rate of remodeling is defined by cells of the osteoblast lineage, which, in addition to bone formation, are also responsible for the activation and recruitment of osteoclast precursors [31]. A

Table 2. Gingival crevicular fluid alkaline phosphatase (IU/L) at the control and experiment site during different time points.

Time Points	Control Site Mean \pm SD	Experiment Site Mean \pm SD
Hour 0	34.25 \pm 11.62	39.45 \pm 10.89
Hour 1	38.50 \pm 11.48	39.75 \pm 11.06
Day 1	38.00 \pm 12.18	41.60 \pm 13.39
Day 7	38.75 \pm 10.75	45.55 \pm 11.83
Day 14	43.00 \pm 11.52	56.75 \pm 11.50*
Day 21	42.65 \pm 11.94	52.40 \pm 11.24

*statistically significant

**Figure 2.** Gingival crevicular fluid alkaline phosphatase (IU/L) at the control and experiment site during different time points.

sequence characterized by periods of activation, resorption, reversal, and formation has been described as occurring in both tension and compression tooth sites during orthodontic tooth movement.

In orthodontics, mechanical stress appears to evoke biochemical and structural responses in a variety of cell types in vivo and in vitro [25,32,33]. The early phase of orthodontic tooth movement involves an acute inflammatory response, characterized by periodontal vasodilation and migration of leukocytes out of periodontal ligament capillaries [2]. The mechanism of bone resorption might also be related to the release of inflammatory mediators that can be detected in the gingival crevicular fluid [25,31].

Increase in the volume of gingival crevicular fluid has been shown at the experimental sites compared with the control sites. This observation is in agreement with earlier reports on the gingival crevicular fluid flow rate during orthodontic tooth movement [34,35]. However, the assessment of clinical parameters such as gingival inflammation in longitudinal studies can be a potential source of bias because examiner subjectivity may lead to drift in the interpretation of assessment criteria over extended periods [36]. Meticulous oral hygiene has been emphasized by thorough oral hygiene practices and periodic examination and/or scaling to control the gingival inflammation in the present study. Also periodic random examination was carried out to ascertain the oral

hygiene maintenance. The increased gingival crevicular fluid flow reflects the changes in the deeper periodontal tissues of alveolar bone and periodontal ligament induced by orthodontic treatment [35,37-39]. The assessment of the volume of gingival crevicular fluid appears to represent a non-invasive method of investigating such clinically unapparent changes.

Analysis of the enzymes associated with bone metabolism such as alkaline phosphatase could be indicators to explain the mechanism. The alkaline phosphatase activity in the gingival crevicular fluid can be influenced by alveolar bone turnover events and therefore reflects metabolic changes in the periodontium [20,40]. Alkaline phosphatase is commonly associated with bone metabolism with osteoblasts showing high levels of these enzymes [19,41]. As a result of orthodontic force application, the enzymes produced in the periodontium diffuse into the gingival crevicular fluid. Thus monitoring of phosphatase activities in the gingival crevicular fluid could be suggestive of the tissue changes occurring during orthodontic tooth movement. Earlier studies [21,23] have shown an increase in gingival crevicular fluid alkaline phosphatase level during orthodontic tooth movement. In the present study, the alkaline phosphatase level was estimated at different time intervals from zero hour to 21 days. At the experiment side, the alkaline phosphatase level showed maximum level on the 14th day after activating the appliance. This observation is in agreement with the previous research [42,43]. Batra [23] also found a decrease in the level of alkaline phosphatase on the 21st day in the experiment side which is in agreement with the present study. This could be attributed to the response of the periodontal structures to the orthodontic forces over time.

In the present study, the duration of the study was 21 days and the time interval of collection for gingival crevicular fluid was scheduled so as to identify and to understand the enzymatic changes occurring during the early stages of orthodontic tooth movement and to coincide with the initial and lag phases of tooth movement [44]. The immediate changes occurring in the periodontium and the rapid tooth movement within the periodontal ligament space were best estimated by finding out the enzyme activity immediately after canine retraction and the first day of force application. The 7 days enzyme activity is expected to coincide definitively with the lag phase of tooth movement as the hyalinization sets in, and the 14 and 21 days enzyme activity are pointers to the continuity of this phase or the beginning of the post lag phase. Considering the above facts, the time interval of this study was selected as pretreatment, 1 hour, 1 day, 7 days, 14 days and 21 days [23]. The peak alkaline phosphatase activity was observed on the 14th days, followed by decline on the 21st days. This implies that the alkaline phosphatase activity followed the rate of tooth movement during the initial phases.

The osteogenic cells in the periodontal ligament respond to the tensional forces with an increase in the maturation rate. In the periodontal ligament, the fibroblast proliferation and collagen has been shown to increase in the tension sites. In addition, the osteoprogenator cell pool responds by increased proliferation and differentiation. The second messengers thus transmit the responses from the periodontal ligament fibroblasts to the osteogenic cells. The bone remodeling process is more complex with resorptive activity initially (3 - 5 days) and is followed by its reversal (5 - 7 days).

Subsequently, a late phase of bone deposition (7 - 14 days) occurs in both tension and pressure sites of the alveolar wall. In the early phases, bone resorption is more than bone deposition, but in the later phase, resorption and deposition become synchronous [40].

The observations of the current study suggest that alkaline phosphatase activity could possibly be a biological indicator of the activity in the periodontium and therefore orthodontic tooth movement.

Conclusion

From the observations of this study it can be concluded that

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