**INTRODUCTION**

Orthodontic therapy aims at the correction of dental irregularities and disharmony in jaw relations. It utilizes the potential of the periodontal ligament and the alveolar bone to adapt to changing mechanical circumstances by tissue modelling. By these adaptations teeth can be moved through the alveolar bone and also distant skeletal locations can be affected. By these two mechanisms, a stable occlusion and proper jaw relation can be established. The basis for this approach is interplay between applied forces, bone deposition, bone resorption, and periodontal ligament remodelling (Alsayagh and Ismael 2011).

Dental pulp, periodontal ligament (PDL), alveolar bone, and gingiva, when exposed to varying degrees of magnitude, frequency, and duration of mechanical loading, express extensive macroscopic and microscopic changes. Orthodontic tooth movement is uniquely characterized by the abrupt creation of compression and tension regions in the PDL (Goutoudi, Diza et al. 2004). Orthodontic tooth movement can occur rapidly or slowly, depending on the physical characteristics of the applied force, and the size and biological response of the PDL (Dongari-Bagtzoglou and Ebersole 1996). These force-induced strains alter the PDL’s 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 favourable microenvironment for tissue deposition or resorption (Simontet, Lacey et al. 1997; Cetin, Buduneli et al. 2004).

The present trend in clinical medicine leans towards the use of noninvasive procedures that determine the changes in salivary constituents to diagnose several diseases. The potential diagnostic importance of gingival crevicular fluid (GCF) was recognized more than 60 years ago (Cimasoni 1983). GCF contains inflammatory products, bacterial products, and products of tissue breakdown. Thus, examination of GCF is an ideal method of evaluating the tissue destruction. Analysis of the gingival crevicular fluid provides a noninvasive method of studying the host response factors in the periodontium (Brill N and B 1958; Embery and Waddington 1994). Binder demonstrated a strong positive relationship between the
levels of the enzymes in GCF and previous disease activity (Binder, Goodson et al. 1987).

During orthodontic tooth movement GCF levels of IL-1β, IL-6, and TNF-α as well as TGF-β and epidermal growth factor (ECF) have all been observed to rise compared with contralateral control sites (Lowney, Norton et al. 1995). This pattern is also reflected in the levels of prostaglandin E2 (PGE2), recognized as a pro-inflammatory mediator of bone metabolism, which is significantly increased following initial onset of mechanical force application (Grieve, Johnson et al. 1994), and the levels of alkaline phosphatase, which is critical in bone deposition was also significantly elevated during tooth movement (Insoft, King et al. 1996). Studies on extracellular matrix components within PDL revealed that the proteoglycan metabolism in GCF is a strong biomarker for assessing bone resorption and remodeling during orthodontic tooth movement (Perinetti, Paolantonio et al. 2002). During orthodontic treatment, acid and alkaline phosphatase, in human GCF, has been correlated with the total appliance duration (Perinetti, Paolantonio et al. 2002).

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. The mechanism of bone resorption might also be related to the release of inflammatory mediators that can be detected in gingival crevicular fluid (GCF). Moreover, orthodontic force involves an increased proliferation and differentiation of the cells of the periodontal ligament into osteoblasts (Krishnan and Davidovich 2006).

In recent years, a number of GCF constituents have been shown to be diagnostic markers of active tissue destruction in periodontal diseases, but only a few studies have focused on the GCF constituents involved in bone remodelling during orthodontic tooth movement (Lamster 1992; McCulloch 1994).

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 (Reitan 1967; Rygh 1972; Storey 1973; Rygh 1976). During orthodontic treatment, acid and alkaline phosphatase in human GCF have been correlated with the total appliance duration (Insoft, King et al. 1996).

The objective of this study was to evaluate and compare GCF ALP activity during orthodontic canine movement with closed coil spring and elastic chain in human subjects as it relates to the time of treatment and the type of stress exerted on the periodontium (tension or compression) by the tooth movement.

**SUBJECTS AND METHODS**

A total of twenty three male orthodontic patients, (age range, 12.8 - 22.3 years; mean 15.5 years) with Class II tending dental malocclusions who required the extraction of the maxillary first premolars and retraction of the maxillary canines as part of their fixed orthodontic appliance treatment were included in the study.

The following inclusion criteria were observed: (1) need for bilateral maxillary first premolars extractions (necessitating moderate anchorage requirements) and fixed appliance orthodontic treatment; (2) similar degree of minimal crowding on each side of the maxillary arch; (3) eruption of second molar with or without the presence of unerupted third molars; (4) no previous orthodontic or orthopedic treatment; (5) no craniofacial anomaly present; (6) no previous reported or observed dental treatment of maxillary canines; (7) no history of trauma, bruxism or parafunctions; (8) no past or present signs and symptoms of periodontal disease; (9) no radiographic evidence of periodontal bone loss; (10) no significant medical history or medication that would adversely affect the development or structure of the teeth and jaws and any subsequent teeth movement; (11) blood studies to rule out any blood dyscrasias; (12) no use of anti-inflammatory drugs during the month preceding the study (Grieve, Johnson et al. 1994; Uematsu, Mogi et al. 1996); (13) probing depth values not exceeding 3 mm in the whole dentition; and (14) a full-mouth plaque score and a full-mouth bleeding score less than or equal to 20%.

All patients were informed about the nature and objectives of the study and their full signed consent (from the patient or his guardian) was obtained prior to entry into the study. The study complied with the rules set by the International Conference on Harmonization of Good Clinical Practice Guidelines, and the Declaration of Helsinki, the protocol was reviewed and approved by the Ethical Committee of the Qassim University College of Dentistry.

In each patient, the maxillary canines undergoing orthodontic treatment were used as the test teeth (TT) and referred group I (G I). Both of the TT canines were distalized into first premolars sites. One TT antagonist mandibular canine (AC) was used as a control and referred group II (G II).

At the commencement of treatment, standard orthodontic records were taken including; extra-oral and intra-oral photographs, alginate impressions and a lateral cephalometric radiograph and an orthopantomograph. Gingival crevices depth was measured, gingival and plaque indices were scored.

**Orthodontic Treatment**

All patients were fitted with upper 0.022 x 0.028 inch slot SPEED19 with upper 0.019 x 0.025 inch β titanium alloy20 arch wire was left in situ for eight weeks. Following extraction of the maxillary first premolars Orthodontic brackets2 were placed on the buccal surfaces of the teeth in the maxillary arch, including incisors, canines and first molars, initial alignment was attained utilizing an 0.014/0.016 inch NiTi wire3 taking an average of 1.9 months (range 0.5-6 months). Following this, an 0.019 x 0.025 inch β Titanium Molybdenum Alloy3 arch wire was left in situ for eight weeks to obtain standardized ligation during canine retraction, thereby regulating the effects of friction.

The distal retraction of the maxillary canines was performed on...
a continuous 0.020 inch stainless steel arch wire\(^5\) to reduce frictional effects. Each patient received pre-calibrated three millimeter superelastic NiTi closing coil spring\(^6\) activated 300\% of its activation, delivering an approximate force of 150g. The coil springs were attached to the maxillary first molar tube\(^7\) and canine brackets\(^8\) on each side via a six millimeter power arm constructed of 0.016 x 0.016 inch stainless steel\(^9\) inserted into the auxiliary tubes of the respective brackets. This was aimed at delivering the force as close to the centers of resistance of the respective teeth as was clinically viable, with the force vector being parallel to the main arch wire. No reactivation of the closing coils was performed.

During canine retraction, participants were recalled every fourteen days for clinical assessments. At each appointment, oral hygiene was reinforced and the appliances were assessed for any damage. As a quality control measure, if a bracket, arch wire or spring involved in canine retraction was damaged, or if the canine attained complete retraction prior to the end of the study period, the patient was excluded from the study. Three patients were excluded for these reasons, bringing the total sample size from twenty three to twenty.

The study period extended from the beginning of canine retraction through a period of 84 days (12 weeks). This ensured that the devices remained active for the entire period of the study. The canine retraction force was checked every appointment with a force gauge (Teclock Co, Nagano, Japan). We confirmed that constant force was produced throughout the experimental period.

**Crevices depth measurements, Plaque index and gingival index scoring**

Full-mouth plaque score and full-mouth bleeding score were recorded as the percentage of tooth surfaces with the presence of supragingival plaque or bleeding within 15 seconds after probing with a 20 g controlled force periodontal probe also measurement of the gingival crevices depth was performed.

**GCF Sampling**

All GCF samples were collected in the forenoon (at same time, of the day) (between 10 and 11 AM) to allow for the circadian variation seen in GCF volume (Bissada, Schaffer et al. 1967). Each crevicular site included in the study was isolated with cotton rolls. Before the GCF collection, any supragingival plaque was removed with cotton pellets, and a gentle air stream was directed toward the tooth surface for 5 seconds to dry the area. A calibrated volumetric micropipette of 5 L capacity was introduced into the gingival crevices of the selected site for collection of gingival crevicular fluid by Brill technique (Brill N and B 1958). The sample was collected for 20 min from gingival crevices of teeth subjected to orthodontic forces and from the mandibular teeth without orthodontic appliance (control sites). Every sample either from the orthodontically treated teeth or from normal teeth was pooled from the mesial and distal sites of the teeth. The GCF sampling procedures and scoring of plaque index, gingival index and crevices depth measurements were done for the study group and the control group at baseline, 4 weeks, 8 weeks and 12 weeks. The collected sample was then transferred to a sterilized plastic vial containing distilled water. The vial was frozen to –20°C until the sample was transported to the laboratory for analysis.

**Laboratory analysis**

Enzyme (kinetic) analysis of the alkaline phosphatase level in GCF was done using a spectrophotometer. The rate of formation of p-Nitrophenol is measured as an increase in absorbance of 405 nm wavelength light which is proportional to the alkaline phosphatase activity in the sample; the spectrophotometric analysis was performed using a semiautoanalyzer. The final results were expressed as corrected optical densities or enzyme units, where 1 unit is equal to 1 mol p-nitro phenyl phosphate converted to p-nitro phenol (colorless) plus inorganic phosphate per minute at the pH and temperature indicated for alkaline phosphatase.

The sample was incubated at 30°C, with less than 0.05°C fluctuation, for 20 minutes in a substrate containing p-nitrophenyl phosphate (10 mmol/L), carbonate buffer (pH 10.2 ± 0.1 at 30°C), mannitol (200 mmol/L), and MgCl\(_2\) (3 mmol/L), to a total volume of 1.0 mL. 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. We used 18.45 as the GCF alkaline phosphatase values in different study line phosphatase activity in the sample; the and plaque index. Perasson’s (1967) analysis (Bissada, Schaffer 1967) was introduced into the gingival crevices of the selected site for collection of gingival crevicular fluid by Brill technique (Brill N and B 1958). The sample was collected for 20 min from gingival crevices of teeth subjected to orthodontic forces and from the mandibular teeth without orthodontic appliance (control sites). Every sample either from the orthodontically treated teeth or from normal teeth was pooled from the mesial and distal sites of the teeth. The GCF sampling procedures and scoring of plaque index, gingival index and crevices depth measurements were done for the study group and the control group at baseline, 4 weeks, 8 weeks and 12 weeks. The collected sample was then transferred to a sterilized plastic vial containing distilled water. The vial was frozen to –20°C until the sample was transported to the laboratory for analysis.

**RESULTS**

Table (1) shows the levels of alkaline phosphatase in the study group (G I) and the control group (G II) and differences between the two groups.

<table>
<thead>
<tr>
<th>Date</th>
<th>Mean ± SD</th>
<th>Group I (n=20)</th>
<th>Group II (n=20)</th>
<th>St. error</th>
<th>P value &lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>865.30±103.44</td>
<td>783.32±92.21</td>
<td>24.213 0.215</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 weeks</td>
<td>1623.85±97.13</td>
<td>792.43±111.21</td>
<td>20.112 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 weeks</td>
<td>1403.66±112.32</td>
<td>813.63±156.32</td>
<td>21.654 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 weeks</td>
<td>1101.82±112.43</td>
<td>799.54±142.16</td>
<td>25.873 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value (ANOVA)</td>
<td>0.001 0.151</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The statistical test compared the two groups together, total alkaline phosphatase levels in gingival crevicular fluid were found to be lowest in baseline measurements in G I, by ANOVA there was a statistically significant difference in the
alkaline phosphatase levels measured during study periods in G I. Considering the differences in alkaline phosphatase level between G I and G II; there was a highly significant difference between the two groups at the 4th, 8th and 12th weeks (P<0.001) with a non-significant difference at baseline (p>0.05) between both groups.

Table (2) shows the Pairwise inter group comparison of GCF ALP levels in group I. There was a statistically significant increase regarding the level of alkaline phosphatase in group I along the duration of the study.

Table (2) Pairwise inter group comparison of GCF ALP levels in group I

<table>
<thead>
<tr>
<th>Pairwise Comparison</th>
<th>Mean difference Bet. 2 periods</th>
<th>Std. error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline - 4weeks</td>
<td>-755.35</td>
<td>14.887</td>
<td>0.001</td>
</tr>
<tr>
<td>Baseline - 8weeks</td>
<td>-538.16</td>
<td>3.124</td>
<td>0.001</td>
</tr>
<tr>
<td>Baseline - 12weeks</td>
<td>-236.32</td>
<td>12.935</td>
<td>0.001</td>
</tr>
<tr>
<td>4weeks - Baseline</td>
<td>758.35</td>
<td>17.546</td>
<td>0.001</td>
</tr>
<tr>
<td>4weeks - 8weeks</td>
<td>220.19</td>
<td>12.965</td>
<td>0.001</td>
</tr>
<tr>
<td>4weeks - 12weeks</td>
<td>522.03</td>
<td>10.342</td>
<td>0.001</td>
</tr>
<tr>
<td>8weeks - Baseline</td>
<td>538.16</td>
<td>11.765</td>
<td>0.001</td>
</tr>
<tr>
<td>8weeks - 4weeks</td>
<td>-220.19</td>
<td>8.675</td>
<td>0.001</td>
</tr>
<tr>
<td>8weeks - 12weeks</td>
<td>301.84</td>
<td>11.923</td>
<td>0.001</td>
</tr>
<tr>
<td>12weeks - Baseline</td>
<td>236.32</td>
<td>9.765</td>
<td>0.001</td>
</tr>
<tr>
<td>12weeks - 4weeks</td>
<td>-522.03</td>
<td>13.754</td>
<td>0.001</td>
</tr>
<tr>
<td>12weeks - 8weeks</td>
<td>-301.84</td>
<td>11.621</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table (3) Spearman rank correlation between GCF ALP levels, gingival index and plaque index

<table>
<thead>
<tr>
<th>Date</th>
<th>ALP level</th>
<th>Gingival Crevice depth</th>
<th>Plaque Index</th>
<th>Gingival Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R value</td>
<td>R value</td>
<td>R value</td>
<td>R value</td>
</tr>
<tr>
<td>Baseline</td>
<td>1.000</td>
<td>0.509</td>
<td>0.045</td>
<td>0.054</td>
</tr>
<tr>
<td>4 weeks</td>
<td>1.000</td>
<td>0.477</td>
<td>0.033</td>
<td>0.076</td>
</tr>
<tr>
<td>8 weeks</td>
<td>1.000</td>
<td>0.436</td>
<td>0.048</td>
<td>0.087</td>
</tr>
<tr>
<td>12 weeks</td>
<td>1.000</td>
<td>0.367</td>
<td>0.091</td>
<td>0.101</td>
</tr>
</tbody>
</table>

Table (3) shows the Spearman rank correlation analysis between GCF ALP levels, gingival index, plaque index and gingival crevice depth. Alkaline phosphatase levels were found to be positively correlated with gingival crevice depth and gingival index. However, there was no correlation found between alkaline phosphatase levels and plaque index P>0.05.

DISCUSSION

In periodontal diseases tissue destruction is seen as the consequence of bacterial interaction, which is due to host cells (mainly polymorphonuclear leukocytes) releasing their granular enzymes (lysozyme, β-glucuronidase) that are capable of attaching to all extracellular matrix components that seem to play an important role in the tissue damage (Cohn and Hirsch 1960; Gustafsson and Nilsson 1961; Van Dyke, Lester et al. 1993).

GCF is an exudate from the microcirculation around the periodontium and gingiva. It picks up enzymes and other molecules that participate in the disease process, immune mechanism, bacterial products, as well as products of cell and tissue destruction (Brill N and B 1958; Cimasoni 1983). The main cells that contribute to the constituents of GCF are polymorphonuclear lymphocytes, macrophages, and plasma cells (Embery and Waddington 1994). Unlike serum and saliva that are the commonly used sources for assessment of biomarkers, GCF is site specific, conveniently sampled, and contains components derived from both the host and the bacteria. Conversely only extremely small volume of fluid is available from a single site, and so GCF needs highly sensitive techniques for quantitative analysis (Cabrini and Carranza 1991; Bissada, Schaffler et al. 1967; Chapple, Matthews et al. 1993; Malhotra, Grover et al. 2010).

Several substances have been found in GCF, these constituents include physiologically active substances and enzymes such as prostaglandins(Offenbacher, Odle et al. 1986; Nakashima, Roehrlich et al. 1994), collagenase(Golub, Siegel et al. 1976), hydroxyproline(Svanberg 1987), β-glucuronidase(Lamster, Vogel et al. 1985), lactate dehydrogenase, glycosaminoglycans (Last, Stanbury et al. 1985), aspartate aminotransferase(Persson, DeRouen et al. 1990), and alkaline phosphatase (ALP)(Chapple, Matthews et al. 1993; Nakashima, Roehrlich et al. 1994; Perinetti, Paolantonio et al. 2008).

Ishikawa and Cimasoni; 1970 first recognized the potential of alkaline phosphatase as an important biochemical component of GCF (Ishikawa and Cimasoni 1970). The sources of ALP are polymorphonuclear leukocytes (PMNL)(Cohn and Hirsch 1960), bacteria within the dental plaque(Bowen 1961) and osteoblasts and fibroblast cells(Cabrini and Carranza 1951). Binder et al.; 1987 demonstrated a strong positive relationship between the levels of alkaline phosphatase in GCF and tissue destruction (Binder, Goodson et al. 1987).

The GCF serum ratio of ALP for clinically healthy periodontal tissues ranged from 6:1 to 12:1, which implies that the enzyme is locally produced within the periodontium (Daltaban, Saygun
et al. 2006). Thus, gingival crevicular fluid ALP levels from gingival crevices can be taken as indicative for active tissue destruction in the periodontium.

Chapple et al.;1996 noted that total GCF ALP levels increased before increases in the gingival index and appears to be a good marker of gingival inflammation. They stated that majority of ALP in GCF is of PMNL origin (Chapple, Socransky et al. 1996). Nakashima et al. 1994 found in their study that total amounts of ALP were significantly higher in periodontitis as compared to healthy and gingivitis sites (Nakashima, Roehrich et al. 1994).

There are abundant PMNL in the site of periodontal inflammation and they are a prime source for GCF ALP (Chapple, Glenwright et al. 1994; Chapple, Socransky et al. 1996; Malhotra, Grover et al. 2010). The decrease in the inflammation in the periodontium resulted in the statistically significant decrease of GCF ALP values (Kunjappu, Mathew et al. 2012). Ishikawa and Cimasoni; 1970 reported that there is a positive correlation between levels of ALP and probing depth and gingival index (Ishikawa and Cimasoni 1970).

Bowen; 1961 reported that there was a lack of correlation of ALP levels with the plaque index, this may be attributed to the fact that even though the plaque bacteria are a source of alkaline phosphatase but the percentage contribution to the total GCF ALP levels is not significant (Bowen 1961), as Chapple; 1996 found that plaque assays have suggested that bacterial ALP contribute less than 20% to total ALP content of GCF (Chapple, Socransky et al. 1996). This confirms that the total ALP level reflects the state of the periodontium more accurately in health and disease and is not significantly affected by the external bacterial factors in plaque (Chapple, Glenwright et al. 1994; Chapple, Socransky et al. 1996).

CONCLUSION

Total alkaline phosphatase levels in gingival crevicular fluid can be used as a diagnostic biomarker to assess the health and pathology of the periodontium during orthodontic treatment. It can be used in early detection of changes in the periodontium and can assess the efficacy and prognosis of orthodontic treatment.

Recommendation

However, since there are multiple sources of ALP in GCF further studies on evaluation of ALP from single source (isoenzyme studies) may increase the diagnostic value of alkaline phosphatase level as a bone destruction maker.

References


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