"Acid Phosphatase activity in Gingival Crevicular Fluid — A non-invasive adjunct in assessing orthodontic tooth movement??"

Puneet Batra* / O. P. Kharbanda** / Ritu Duggal*** / Neeta Singh**** / Har Parkash*****

Acid phosphatase (ACP) activity in the gingival crevicular fluid (GCF) plays an important role in understanding orthodontic tooth movement as it is associated with bone metabolism. The enzyme activity is high in the osteoclastic phenotype. The aim of the study was to investigate whether this enzyme can be used as a non-invasive marker and diagnostic adjunct in orthodontics. 10 female patients requiring all first premolar extractions were treated with Standard Edgewise mechanotherapy. Canine retraction was done using 100gm tension springs with one maxillary canine acting as active site and the other acting as control. GCF was collected from mesial and distal aspects of canines before initiation of canine retraction, immediately after initiation of retraction, 1st, 7th, 14th and 21st day after initiation of retraction. The results show significant (p<0.05) changes in enzyme activity on the 7th and 14th day on both mesial and distal aspects of the active and control sites. The study showed that ACP activity can be successfully estimated in the GCF. The peak in enzyme activity occurred on the 14th day of initiation of retraction. The duration of pre-retraction levelling and alignment phase was shown to influence the initial enzyme activity. Thus assay for ACP activity can give the clinician a non-invasive adjunct in evaluating treatment progress, in addition to the clinical findings.

Key words: Abominators, Phosphatase, Osteoclasts, Lag phase, Canine retraction, Constant force

Introduction

Gingival crevicular fluid (GCF) is regarded as a promising medium for detection of factors associated with changes in the underlying periodontium due to orthodontic force application. In recent years, it is suggested that enzyme content of gingival crevicular fluid could play an important role in understanding orthodontic tooth movement. GCF is an osmotically mediated inflammatory exudate that is found in the gingival sulcus. As an exudate, the amount of fluid in any crevicular location tends to increase with inflammation and capillary permeability. Serum is the primary source of the aqueous component of the GCF.1-8

The biological response incident to orthodontic tooth movement ultimately involves alterations in the surrounding bone architecture.9-11 Resorbing cells such as osteoclasts and macrophages, have been shown to have high Acid Phosphatase activities12. Acid phosphatase is present in lysosomes which are present in all cells except erythrocytes. The greatest concentrations of extralysosomal activity occur in liver, spleen, erythrocytes, platelets, bone marrow and prostate. Acid phosphatase activity may be useful in diagnosing carcinoma of the prostate, Pagets disease, hyperparathyroidism, Gauchers disease, Newmann Picks disease, Myelocytic leukemia and some hematological disorders.13

Acid and Alkaline Phosphatases are released by injured or damaged, or dead cells into extracellular tissue fluid. As a result of orthodontic force application, these enzymes produced in the periodontium diffuse into the gingival crevicular fluid. The concentration of
these enzymes can readily be assayed from this gingival crevicular fluid. The monitoring of phosphatase activities in the gingival crevicular fluid could be clinically useful as a biological monitor, of the tissue changes occurring during orthodontic tooth movement. In an experimental study in rats, Stephan suggested that phosphatase activities reflect bone turnover in orthodontically treated tissues. In an in vitro study Michael Insoft, King and Keeling correlated alveolar bone remodeling with changes in gingival crevicular fluid phosphatase activities. In this study early elevation in alkaline phosphatase activity was observed during a time when little tooth movement occurred i.e. between 1\text{st} and 3\text{rd} week. Elevation in acid phosphatase activity occurred during the period of peak tooth movement i.e. between 3\text{rd} and 6\text{th} week. These trends support observations made from histological studies and suggest that changes in gingival crevicular fluid phosphatase activities may reflect local biologic processes associated with orthodontic tooth movement.

There exists a temporal and spatial difference in phosphatase activity in GCF from orthodontically treated and control sites in the same individual. Thus it is imperative to adapt commercially available sensitive assays to achieve accurate measurements of acid and alkaline phosphatase activities in GCF and to use these assays to monitor phosphatase activities temporally and spatially in human subjects.

This study was undertaken to assess alteration in the acid phosphatase enzyme activity during orthodontic tooth movement. To estimate and compare the activity acid phosphatase in gingival crevicular fluid before and during application of orthodontic force. It was undertaken with the goal of exploring the possibility of using this enzyme as a non invasive biological marker and diagnostic aid in orthodontics.

Material and methods:

10 female patients requiring orthodontic treatment with all first premolar extractions were taken up for the study. The patients were in permanent dentition and good periodontal and general health. The patients were treated with conventional standard Edgewise (.022 x .028) mechanotherapy. Canine retraction was done using self-ligating spring with 100 grams of constant force. The gingival crevicular fluid was collected from the mesial and distal side of maxillary canines. Retraction of canine was initiated on one side with the other side acting as control. The gingival crevicular fluid was collected before canine retraction was initiated, immediately after initiating canine retraction (0 day), 1\text{st}, 7\text{th}, 14\text{th} and 21\text{st} day after initiation of canine retraction.

The following criteria were taken into consideration for case selection:

1) Good general health (assessed after careful history taking)
2) Patients were without any systemic disease during study period.
3) Gingiva showed no sign of inflammation with a plaque and gingival index of £ 1.
4) Good periodontal health with generalized probing depths less than 3 mm
5) No radiographic evidence of periodontal bone loss.
6) Patients ruled out for pregnancy and other hormonal changes.

Patient preparation

The patients were treated with Conventional Standard Edgewise mechanotherapy (.022 x .028 slot). Canine retraction was done on .018 X .025 stainless steel wire. In all cases canine retraction was begun on the right maxillary canine (active site). No force was applied on the left maxillary canine (control site) during the experimental period. Canine retraction was done using self-ligating closed coil spring (9mm) of 100 gms force manufactured by GAC (Central Islip, N.Y.).

Experiment design

Premolar extractions were carried out at least three months prior to collection of gingival crevicular fluid. Thorough oral prophylaxis was done one week prior to collection of samples. Strict oral hygiene instructions were given. To ensure optimal control of bacterial plaque, each patient was asked to rinse twice daily with 0.5 ounces of 0.2%chlorhexidine gluconate throughout the study period.

Patients were not allowed to take any medications or drugs (NSAIDS) during the study to avoid any interference in the inflammatory process masking the tissue reactions and ultimately enzyme activities.

Gingival Crevicular Fluid Collection

Gingival crevicular fluid was collected using volumetric micropipettes of 1ml capacity (obtained from Borosil company limited, USA).

The patients were asked to gargle vigorously with a glass of sterile water to cleanse the oral cavity. After proper isolation the micropipette was placed extracrevicularly and 1ml of GCF was collected from mesial and distal sides of the active and control sites. In case of inadequate sample,
collection was done twice or thrice until 1ml was obtained.

Sample Preparation

1ml of GCF was made to 100ml with Sorensens medium containing 0.05% bovine serum albumin in phosphate buffered saline (pH= 7.0). The samples were collected in eppendorf tubes, which were properly labelled prior to sample collection and put on ice. The samples were taken to the biochemistry laboratory immediately. The samples were centrifuged at 2000 rpm in a refrigerated microcentrifuge for one minute to remove the bacterial and cellular debris. If samples could not be immediately analyzed a drop of acetic acid stabilizer was added to the samples. These samples were then stored at −70°C until assayed for enzyme activities.

Acid Phosphatase Estimation

The enzyme activity was analyzed using the commercially available quantitative kit obtained from Boehringer Mannheim, Germany.

The principle of the reaction is as follows:

\[
\text{acid} \
\text{1-naphthyl phosphate + H}_2\text{O} \rightarrow \text{phosphate + 1-naphthol} \
\text{naphthol + 4-chloro-2-methylphenyl diazonium salt} \rightarrow \text{azo dye}
\]

(Colored product)

Composition in the principle reactions:

- Citrate buffer (135 mmol/L, pH= 4.8)
- 1-Naphthyl phosphate (11 mmol/L), Fast red TR – salt (1.1 mmol/L)
- 1,5- Pentane diol (200 mmol/L)
- Sodium taartrate (90 mmol/L)
- Acetic acid (3 mmol/L)

If stabilizer was added to the sample it was stable for 3 days at +4°C and for 24 hours at +15 to 25°C. So sample was preferably stored at −70°C.

Estimation Procedure:

The reagent mixture was prepared by dissolving the contents of bottle 1 with 2.0 ml of citrate buffer (bottle 2). 50ml of the sample was taken by a pipetemann in a cuvette to which 0.5ml of the above reagent mixture was added. The control cuvette contained Sorensens medium. The cuvette was placed in the spectrophotometer and readings were noted after 5 min of initiation of the reaction (A1), 1 minute later (A2), 2 minutes later (A3) and 3 minutes later (A4). The absorbance was read at 405nm. Absorbance change was noted by summation of the changes in absorbance over the 3 minute period starting from A1 to A4. (A3-A2) + (A4-A3). The change in absorbance was designated as delta A. Mean change in absorbance per minute was calculated (delta A / min).

Total acid phosphatase was calculated using the formula U/L = 643 X delta A / min.

Results:

The quantitative estimation of enzyme levels of Acid Phosphatase was carried out in unit per liter (IU/L). The statistical analysis was assessed as mean level of activity, standard deviation of the mean values of each enzyme at all the sites, ANOVA, LSD multiple comparison test and Pearson correlation coefficient.

Enzyme activity on mesial surface of active site

The average pretreatment basal acid phosphatase activity (1.523 IU/L) on the mesial surface was slightly more as compared to the day 0 value (1.436 IU/L) on that site. There was a progressive fluctuation in enzyme activity from day 0 to day 21 with enzyme activity decreasing and then increasing alternatively on day 1, 7, 14 and 21. On the 1st day there was decrease in activity by 0.2 folds (1.2 IU/L). Subsequently a 1.25 fold increase in activity was observed on the 7th and a slight decrease in activity on the 14th day (1.5 IU/L and 1.458 IU/L respectively). This was followed by a 1.2 fold increase in enzyme activity on the 21st day. However the enzyme activity remained higher than the activity observed at the 1st day of initiation of canine retraction as well as the pretreatment values. (Table 1 and Graph 1)

Enzyme activity on mesial surface of control site

The average pretreatment basal acid phosphatase (1.2 IU/L) was lower than the activity at the active site and the day 0 value at the control site (1.479 IU/L). Decrease in activity was observed on the 1st and 7th day (1.458 IU/L and 1.050 IU/L respectively). This was followed by a 1.2 and 1.3 fold rise in enzyme activity at day 14 and 21 respectively (1.286 IU/L and 1.372 IU/L). (Table 1 and Graph 1)

Enzyme activity on distal surface of active site:

The mean pretreatment basal acid phosphatase activity (1.565 IU/L) was higher than the activity at initiation of canine retraction (1.350 IU/L). Subsequent to initiation of canine retraction rise in enzyme activity on the 1st, 7th and 14th day by 1.5, 2.8 and 3.6 folds compared to day 0 values (2.058 IU/L, 3.837 IU/L and 4.801 IU/L respectively). On the 21st day the enzyme activity fell sharply but
remained higher than the activity on the day of initiation of canine retraction (1.522 IU/L). The Newmann-Keuls multiple comparison test at 0.05 significance level showed that pretreatment scores and scores at day 14 and 21 were significant. (Table 1 and Graph 2)

Enzyme Activity on distal surface of control site:

The mean pretreatment basal acid phosphatase activity was 1.050 IU/L. The enzyme activity increased progressively after initiation of canine retraction (1.222 IU/L) to the day 7 score. (1.286 IU/L at day 1 and 1.307 IU/L at day 7). Subsequently enzyme activity showed a slight decrease on the 14th day and remained stable upto the 21st day (1.243 IU/L). (Table 1 and Graph 2).

Comparison of enzyme activity at mesial surface of active and control sites:

The pretreatment basal acid phosphatase activity was higher on the active site as compared to the control site. The enzyme activity on the 7th day showed a significant correlation (p < 0.05) between enzyme scores on the active and control sites. The activity at the active site was 1.5 times the activity compared to corresponding activity on the control site. The enzyme activity on the 14th day was also significantly correlated (p < 0.05) on the active and control sites. The active site activity was 1.1 times the activity at the control site. No significant correlation also occurred between enzyme activity at the active and control sites on the 21st day. (Table 2 & Graph 1)

Comparison of enzyme activity at distal surface of active and control sites:

The pretreatment basal acid phosphatase activity was 1.5 times higher on the active site compared to the control site. However no significant correlation existed between pretreatment scores. At initiation of canine retraction the activity was higher on the active site compared to the control site. No significant correlation existed between day 0 and day 1 scores. The 7th day showed a significant correlation (p < 0.05) between enzyme scores on the active and control sites. The activity at the active site was 3 times the corresponding activity on the control site. The enzyme activity on the 14th day is also significantly correlated (p < 0.05) on the active and control sites. The active site activity is 3.9 times the activity at the control site. No significant correlation also occurs between enzyme activity between the active and control sites on the 21st day. (Table 3 & Graph 2)

Discussion:

A thorough analysis of the GCF can give us valuable information about the underlying changes in periodontium. However, the gingival tissue and the crevice, can modify it. The main advantages of gingival crevicular fluid are site specificity and more reliability to show local changes. By analyzing and estimating the various components of the GCF, especially the enzymes associated with bone metabolism such as acid and alkaline phosphatase can provide immense help and really guide the clinician for better results. Resorbing cells such as osteoclasts and macrophages, have been shown to have high acid phosphatase activity. Therefore the present study was undertaken to reach at a diagnostic correlation between the presence of acid phosphatase enzyme and the remodelling / resorptive changes in the periodontium. 16-21

However at the onset it is emphasised that caution must be exercised while interpreting phosphatase data for specific histological and bony changes. Acid phosphatase enzyme has been shown to be labile under certain environmental conditions and its activity can be excessively high in hemolyzed samples. Moreover, while acid phosphatase activity seems to be a good marker for metabolic bone activity, it is not exclusively found in bone. A closer approximation of bone turnover activity can be obtained by assaying tartrate resistant acid phosphatase. Despite these concerns, it is likely that phosphatase activities in GCF can be influenced by alveolar bone turnover events because serum phosphatase activities associate with bone turnover events in tooth movement, growth and metabolic diseases and as an exudate, GCF reflects metabolic changes in the periodontium. 15

It is critical to understand that though the control was in the same patient and in similar intraoral environment yet the intrabone environment was variable. Thus the pretreatment enzyme activity is not the true basal activity since the dynamics of bone remodeling continued even one month after ligation of canine retraction archwire.

Bone resorption could be frontal or undermining. The application of optimum orthodontic force will results in direct bone resorption on the pressure side. It is produced by the osteoclasts that are formed directly along the bone surface in the area corresponding to the compressed fibers. The duration of the orthodontic tooth movement can be divided into an initial period and a secondary one; direct bone resorption, also called frontal bone resorption, is found notably in the secondary period. Direct bone resorption also occurs along the bone surface adjacent to a compressed hylanized zone. 22
Histochemical localisation of intracellular acid phosphatase is discrete because it is localized mainly in specific membrane bound organelles, the lysosomes. Osteoclasts in bone and odontoclasts in dentin exhibit an intense acid phosphatase activity. The enzyme is localised in the part of the cytoplasm that lies apposed to the resorbing surface of bone and dentin. Electron microscopic studies reveal the enzyme is localised in the lysosomes although activity is also seen extracellularly between the microvilli like projections of the ruffled border. Uptake of resorbed mineral, hydrolyzed collagen fibers and injected radioactive substances at sites of resorption have been observed. Several recent studies imply that acid phosphatase may confer calcifiability to the organic matrix by its hydrolytic action on the protein polysaccharide granules present in the zone of mineralization.

The acid phosphatase activity increased after application of orthodontic force on the distal surface of the active site. The activity on the 21\textsuperscript{st} day showed a sharp fall and reduced to about 1/3 of the activity at day 14 (still activity was 1.1 folds the activity at day 0). However a large inter patient variation is evident when the range was seen. The activity on the 14\textsuperscript{th} and 21\textsuperscript{st} day was statistically significant (p < 0.05). This meant that though acid phosphatase activity showed an initial increase as in other studies yet there was a sustained increase in activity upto 14\textsuperscript{th} day contrary to other studies reported in literature. This highlights that the initial resorptive phase was long and that hyalinization had not set in upto the 14\textsuperscript{th} day. Thus the distalising force was not tissue destructive. The secondary phase of resorption set in after the 14\textsuperscript{th} day.

It is important to apply the initial forces in such a manner as to avoid formation of excessively widespread cell free zones because the duration of resorption will be more or less proportional to the extent of hyalinized zone. The osteoclasts in the PDL are believed to originate from the hemopoietic system in or before the differentiation of the monocyte series. According to Kahn and Malone\textsuperscript{25} dissemination of osteoclasts occurs via blood vascular system. Progenitor osteoclasts are recruited to the sites of bone resorption through chemotaxis. Induction interaction seems to exist between the bone matrix and the precursor cells resulting in the appearance of mature multinucleated osteoclasts. Fibroblasts and macrophages eliminate the hyalinised zone. Thus the orthodontic force in the patients used in this study for canine retraction was near optimal. It can be deduced that osteoclastic mobilisation was rapid and in confirmation with human studies which have shown a mobilisation to take place in the first 20-30 hours of application of force. Besides a delay in hylanisation points to the fact that hylanisation is of the type obtained after application of light force (which shows less destruction compared to that obtained after application of heavy force). Such a hylanised zone can be rapidly removed and rapid tooth movement can be anticipated.

Acid phosphatase activity on the mesial surface of the active site showed fluctuation in activity showing a successive fall and rise on 1\textsuperscript{st}, 7\textsuperscript{th}, 14\textsuperscript{th} and 21\textsuperscript{st} day. This finding again points to the fact that bone remodelling is in a continuous state of flux and the role played by cells are not necessarily discrete. Histological studies are redefining the roles of major cells and newer vistas are being explored. Another fact that enzyme activity was in a constant flux even on the control site shows that remodelling continues even 6 weeks after the insertion of the edgewise archwire. This is in conformance with other studies\textsuperscript{20,21,26,27} which have shown bone resorption continuing even 2 weeks after discontinuation of orthodontic force.

The biochemical indices of bone remodeling like acid and alkaline phosphatases correlates significantly with growth rates (Stephan et al 1985\textsuperscript{28}). The mean age group of the sample was 16.1 years (range 12-21 years). The skeletal maturation of the sample was assessed by using the cervical vertebrae maturation index (CVMI index). Thus 7 patients belonged to the category in which 90% or more skeletal maturation was over. It could be expected that bone dynamics would be faster and more expressive in terms of higher enzyme activities. This was indeed the case, however other patients too showed high values. This broadly implies that other factors like nutritional habits and duration of the leveling phase during treatment and probably other biologic and mechanical variables act in tandem.

Pretreatment enzyme activity was also variable probably due to the vast variability in the duration of leveling. Leveling and precanine retraction mechanics (bite opening and anchorage preparation) were complete by 3.5 months (mean) of beginning of treatment. However the duration of precanine retraction phase varied from 54-174 days (in patient 6 and 1 respectively). It could be thus hypothesised that if the leveling phase took longer time the enzyme activity was less due to settling down of the enzyme activity, which would have risen during leveling. In other words, in patients where leveling was achieved faster the pretreatment enzyme activity was more. However it could be argued that such a claim would be not be substantiated as
alternating peaks and fall in enzyme activity occurs during force application and it is difficult to tell the underlying condition of the periodontium. Still we could easily conclude that in patients where pretreatment and day 0 activity was low faster tooth movement could be expected as the bone was in a favourable dynamic state. It is obvious that high enzyme activity means lesser cellular activity at that site. This statement holds true for the day on which enzyme activity peaks. In the study it was observed that the enzyme activity peaked on the 14th day in most patients. This was followed in most cases by a sharp fall in activity by the 21st day. This fall would depend on the rate of removal of the hylanized zone. This is supported by the study by Yokoya et al. (1997) who stated that on the pressure side osteoclasts increased in number up to the 7th day but fell rapidly by the 14th day.

The socio-economic and the nutritional status of the patient was also assessed. Studies show that enzyme activity is more in subjects consuming more fatty acids. Usually patients belonging to a lower socio-economic background consume a diet, which is poor in proteins, minerals and vitamins, but rich in fats. Fatty acids are precursors of prostaglandins, which play a significant role in bone remodeling (specially E1 and E2 series). Also a vegetarian and non vegetarian diet contain varying amounts of fats. However no correlation between enzyme activity and nutritional pattern was observed in this study. Again this might be due to a multitude of minor factors influencing activity.

Gordon (1993) noticed a correlation between the weight / height ratio and alkaline phosphatase levels in normal healthy subjects. This is interesting fact was noticed in healthy orthodontically treated patients. It was found that the range of enzyme activity (maximum – minimum) correlated with the weight / height ratio. Such a relation could help us to formulate a relation between the above stated ratio and range of enzyme activity. Such a relation could conceivably affect the clinical interpretation of results. However no such relation existed between acid phosphatase activity and the weight / height ratio.

Sandy, Farndale, and Meikle concluded that the osteoblast is now perceived as the cell that regulates both the formative and resorptive phases of the bone remodeling cycle in response to hormonal and mechanical stimuli.

This study helps us in establishing acid phosphatase activities can act as a diagnostic tool for the clinician in deciding the various force levels to be applied during various stages of treatment and also to understand the underlying tissue changes in the periodontium: because of interpatient and intrapatient variation in levels, it may not be possible to have final values as reference. However the pattern of the enzyme change assumes a greater significance and if properly understood is likely to stand as an adjunct to proper treatment assessment. Even though enzyme levels show variation between patients and between same days for a particular enzyme, the similarity of variations produces a ray of optimism for further studies.

This study shows promise for eventual monitoring of biological processes in GCF during orthodontic tooth movement in human subjects. However whether these phosphatase changes are measuring localised alveolar bone remodelling remains to be confirmed with other experimental approaches. Since it is unlikely that correlative bone histology will be forthcoming because of ethical considerations involved, longitudinal studies of orthodontic, monitoring phosphatase activity in the GCF as well as tooth movement and appliance activity may help to clarify these relationship in human subjects. If association exists between important clinical measures and measures of tissue turn over in the GCF, the latter is clinically useful to biologically monitor and predict orthodontic treatment. Such data may aid the orthodontist in assessing other clinically important issues such as patient compliance and appliance management based on individual tissue response. This study also offers question and scope for revisiting what is optimum force?

**Summary and Conclusions**

The following conclusions were derived from this study:

1. Acid phosphatase can be successfully estimated in the GCF during orthodontic tooth movement.
2. There is a definite pattern of variation of the enzyme levels in the GCF in both active and control sites associated with the application of constant force. The peak of enzyme activity usually occurs on the 14th day of force application.
3. Acid phosphatase activity shows inter patient variability in terms of pattern of rise and fall in activity. Thus acid phosphatase activity is not a very reliable representative of the local biological processes taking place in the periodontium.
4. The activity of acid phosphatase in the midst of treatment can give the clinician a non-invasive adjunct in treatment progress evaluation in addition to clinical findings.
Table 1: Mean Acid Phosphatase activity

<table>
<thead>
<tr>
<th></th>
<th>Active site</th>
<th>Control site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mesial surface</td>
<td>Distal surface</td>
</tr>
<tr>
<td>Pre treatment</td>
<td>1.523 ± 0.658</td>
<td>1.565 ± 0.607</td>
</tr>
<tr>
<td>Day 0</td>
<td>1.436 ± 0.750</td>
<td>1.350 ± 0.613</td>
</tr>
<tr>
<td>Day 1</td>
<td>1.200 ± 0.454</td>
<td>2.058 ± 0.853</td>
</tr>
<tr>
<td>Day 7</td>
<td>1.500 ± 0.663</td>
<td>3.837 ± 1.081</td>
</tr>
<tr>
<td>Day 14</td>
<td>1.458 ± 0.450</td>
<td>4.801 ± 1.051</td>
</tr>
<tr>
<td>Day 21</td>
<td>1.779 ± 1.779</td>
<td>1.522 ± 0.666</td>
</tr>
</tbody>
</table>

Table 2: Comparison of Acid Phosphatase activity at Mesial surface of Active and Control sites

<table>
<thead>
<tr>
<th>Days</th>
<th>Active site Mean IU/L±S.D.</th>
<th>Control site Mean IU/L±S.D.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment</td>
<td>1.523 ± 0.658</td>
<td>1.200 ± 0.658</td>
<td>.2102</td>
</tr>
<tr>
<td>Day 0</td>
<td>1.436 ± 0.750</td>
<td>1.479 ± 0.520</td>
<td>.7718</td>
</tr>
<tr>
<td>Day 1</td>
<td>1.200 ± 0.454</td>
<td>1.458 ± 1.115</td>
<td>.0803</td>
</tr>
<tr>
<td>Day 7</td>
<td>1.500 ± 0.663</td>
<td>1.050 ± 0.500*</td>
<td>.0000</td>
</tr>
<tr>
<td>Day 14</td>
<td>1.458 ± 0.450</td>
<td>1.286 ± 0.515</td>
<td>.0000</td>
</tr>
<tr>
<td>Day 21</td>
<td>1.779 ± 1.000</td>
<td>1.372 ± 0.407</td>
<td>.3324</td>
</tr>
</tbody>
</table>

* Ρ < 0.05 is significant

Table 3: Comparison of Acid Phosphatase activity at Distal surface of Active and Control sites

<table>
<thead>
<tr>
<th>Days</th>
<th>Active site Mean IU/L±S.D.</th>
<th>Control site Mean IU/L±S.D.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment</td>
<td>1.565 ± 0.607</td>
<td>1.050 ± 0.635</td>
<td>.2102</td>
</tr>
<tr>
<td>Day 0</td>
<td>1.350 ± 0.613</td>
<td>1.222 ± 0.405</td>
<td>.7718</td>
</tr>
<tr>
<td>Day 1</td>
<td>2.058 ± 0.853</td>
<td>1.286 ± 0.525</td>
<td>.0803</td>
</tr>
<tr>
<td>Day 7</td>
<td>3.837 ± 1.081</td>
<td>1.307 ± 0.489</td>
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<tr>
<td>Day 14</td>
<td>4.801 ± 1.051</td>
<td>1.243 ± 0.503</td>
<td>.0000</td>
</tr>
<tr>
<td>Day 21</td>
<td>1.522 ± 0.666</td>
<td>1.243 ± 0.438</td>
<td>.3324</td>
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</tbody>
</table>

* Ρ < 0.05 is significant
### Table 4: Enzyme activity and related parameters

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (days)</th>
<th>CVM score (% of growth left)</th>
<th>Socio-economic status</th>
<th>Duration of pre-canine retraction phase</th>
<th>Weight / Height Ratio</th>
<th>Acid Phosphatase activity (Max-Min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15 (5-10%)</td>
<td>5</td>
<td>Upper middle class</td>
<td>174-days</td>
<td>64 kgs / 171 cms (0.374)</td>
<td>3.215 IU/L - 0.429 IU/L = 2.786 IU/L</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>6 (little or no growth left)</td>
<td>Upper middle class</td>
<td>71 days</td>
<td>56 kgs / 148 cms (0.378)</td>
<td>4.715 IU/L - 0.643 IU/L = 4.072 IU/L</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>6 (little or no growth left)</td>
<td>Upper middle class</td>
<td>85 days</td>
<td>60 kgs / 164 cms (0.366)</td>
<td>5.144 IU/L - 0.429 IU/L = 4.715 IU/L</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>6 (little or no growth left)</td>
<td>Middle class</td>
<td>135 days</td>
<td>69 kgs / 163 cms (0.423)</td>
<td>5.573 IU/L - 0.643 IU/L = 4.930 IU/L</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>6 (little or no growth left)</td>
<td>Lower middle class</td>
<td>140 days</td>
<td>52 kgs / 154 cms (0.337)</td>
<td>3.644 IU/L - 0.643 IU/L = 3.001 IU/L</td>
</tr>
<tr>
<td>6</td>
<td>19</td>
<td>6 (little or no growth left)</td>
<td>Lower middle class</td>
<td>54 days</td>
<td>65 kgs / 164 cms (0.396)</td>
<td>3.429 IU/L - 0.642 IU/L = 3.000 IU/L</td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td>5 (5-10 %)</td>
<td>Middle class</td>
<td>142 days</td>
<td>70 kgs / 169 cms (0.414)</td>
<td>4.930 IU/L - 0.643 IU/L = 4.287 IU/L</td>
</tr>
<tr>
<td>8</td>
<td>13</td>
<td>3 (25-65%)</td>
<td>Middle class</td>
<td>81 days</td>
<td>67 kgs / 154 cms (0.435)</td>
<td>5.787 IU/L - 0.643 IU/L = 5.358 IU/L</td>
</tr>
<tr>
<td>9</td>
<td>13</td>
<td>3 (25-65%)</td>
<td>Lower middle class</td>
<td>106 days</td>
<td>42 kgs / 110 cms (0.381)</td>
<td>5.787 IU/L - 0.643 IU/L = 5.358 IU/L</td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td>2 (65-85%)</td>
<td>Poor</td>
<td>67 days</td>
<td>56 kgs / 162 cms (0.345)</td>
<td>6.216 IU/L - 0.843 IU/L = 5.373 IU/L</td>
</tr>
</tbody>
</table>

**Graph 1: Mean acid phosphatase activity on mesial surface**
Graph 2: Mean acid phosphatase activity on distal surface

![Graph showing enzyme activity over time]

References:
16) Lilja ES, Lindskog, Hammerstrom L. Histochemistry of enzymes associated with tissue


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