Detection and assessment of Cathepsin K in gingival crevicular fluid during human orthodontic tooth movement

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Abstract

Introduction: Orthodontic tooth movement causes sequential release of numerous biomarkers from the periodontal tissues, this study helps us to better understand the biological processes involved.

Objectives: This study was designed to detect and assess the Gingival Crevicular Fluid (GCF) levels of lysosomal protease, Cathepsin K during human orthodontic tooth movement.

Materials and Methods: Eight bimaxillary protrusion patients undergoing orthodontic treatment with four first bicuspid extractions were selected. Retraction of the canine was initiated by giving lace back on maxillary right canine which was used as Control Tooth (CT) and maxillary left canine used as Test Tooth (TT) with no laceback. From each patient 4 GCF samples were collected 1 hour before, on 1st day (24 hours), on 7th day(168 hours), and after 1 month(30 days). The dynamics of mechanically stimulated Cathepsin K levels in GCF was assessed using enzymatic immunoassay (ELISA).

Results: Results show significant differences between the control and treated teeth for Cathepsin K, with mean values significantly higher for treated site than control sites. On 7th day, at the test side, the levels of Cathepsin K were higher than the corresponding control sides. Another important finding was seen on the 30th day, where Cathepsin K levels were significantly higher on the control side when compared to the test side.

Conclusion: These results indicate that the amount of Cathepsin K in GCF increased during the initial period of orthodontic tooth movement. However, levels of Cathepsin K can be reliably measured during the acute phase of treatment and beyond that the values are unreliable.

Keywords: Cathepsin K, GCF, ELISA.

Introduction

The existence of gingival crevicular fluid (GCF), a fluid that emerges between the tooth surface and epithelial integument, has been recognized for over 100 years but even today the exact nature of the fluid, its origin and composition is the subject of controversy. This may be due to variations in the amount and nature of the fluid produced under different clinical conditions and also by the use of a wide variety of sampling methods.[1]

Orthodontic tooth movement produces remodeling changes in paradental structures leading to variations in the level biochemicals like cytokines, neurotransmitters, arachidonic acid metabolites etc which are in turn reflected in the GCF of moving teeth.[2] Assessment of these GCF biomarkers is clinically significant because it may lead to better understanding of mechanical stress resulting in shorter treatment time with minimal side effects.

The early phase of orthodontic tooth movement always involves an acute inflammatory response, characterized by periodontal vasodilatation and migration of leucocytes out of the capillaries. [3] These leucocytes produce cytokines that interact directly or indirectly with the adjacent paradental cells. After acute inflammatory response, chronic inflammation prevails until the next clinical activation, thereby starting another period of acute inflammation, which is superimposed on the ongoing chronic inflammation.

Cathepsins are potent proteases found in Lysosomes and get activated in low pH, thus the activation of the Cathepsin family lies within the organelles. Interestingly, exceptions such as Cathepsin K, work extracelluarly after being secreted by osteoclasts as seen during bone resorption.[4] Cathepsin K is the one of the most potent mammalian collagenase and plays a key role in bone remodeling and cartilage breakdown and is used as a well-known marker of osteoclast activity. There are studies which show increased level of Cathepsin K in GCF of patients with periodontitis.[5] Early induction of Cathepsin K mRNA may cause an imbalance in the relative resorption activities on the pressure and tension side.[6]

The role of Cathepsin K in orthodontic tooth movement and its mechanism of action could be made further clear by controlled experimental studies. However, no study has been done to show the expression of Cathepsin K in GCF during human orthodontic tooth movement. The purpose of this study was to find out the presence and to assess the level of Cathepsin K during orthodontic tooth movement.

Materials and Methods

Eight patients between 12 to 24 years needing orthodontic treatment for Angle's Class I bimaxillary protrusion requiring four first premolar extractions as a treatment plan, were selected with following inclusion criteria: good general health status, clinically and radiologically healthy periodontal tissues, probing depth less than 3mm, no

radiographic evidence of periodontal bone loss and nonsmoking individuals.

They were bonded with fixed orthodontic appliance 0.022" (Preadjusted Edgewise Appliance) bracket slot, MBT prescription. Maxillary right canine was used as control tooth (CT) and maxillary left canine used as test tooth (TT). After banding and bonding was completed 0.016" NiTi wire was placed and laceback were given using 0.010 SS ligature wire only on test side with approximate force values of 200cN as measured on a Dontrix gauge.

Sample collection

All patients were given strict oral hygiene instructions to be followed throughout the study period. Patients were instructed not to take any medications or drugs including Non-steroidal anti-inflammatory drugs during the study period. Gingival index and periodontal disease index scores were recorded before the collection of gingival crevicular fluid and the mean gingival index was 0.067±0.017.

64 GCF samples, the sample size determined based on power analysis at a confidence interval of 70%, were then collected in the following manner:-

On test side

From each patient 4 GCF samples were collected 1 hour before, and at 1st day (24 hours), 7th day (168 hours), and 1 month (30 days) respectively after the placement of the orthodontic appliance on maxillary left canines (TT).

On control side

From each patient 4 GCF samples were collected 1 hour before, and at 1st day (24 hours), 7th day(168 hours), and 1 month (30 days) respectively after the placement of the orthodontic appliance on maxillary right canines (CT).

GCF collection

GCF of approximately 2 microliter was collected from the distal sulcus of the canine by extracrevicular method using a graduated micro capillary pipette (Sigma Aldrich, Bangalore, India) and were wrapped in a tin foil, placed in Eppendorff vial, and stored at -80°C in refrigerator (New Brunswick Scientific) until analysis. The GCF samples were retrieved from the pipettes using a blower and the entire volume of collected GCF was transferred directly to the prepared microplate wells.

Biochemical Analysis: Principle Of The Assay

The GCF level of Cathepsin K was assessed using enzymatic immunoassay. ELISA kit was procured from Hysel India Private Limited, Delhi which uses the solid phase enzyme immunoassay technique. A biotin-conjugated antibody specifically made for Cathepsin K is pipetted into the wells. A wash buffer is used to flush the well and Avidin conjugated Horseradish Peroxidase (HRP) is dispensed into the well. Flushing of the well to remove any unbound Avidin-enzyme reagent was followed by addition of a substrate solution for color to develop. A microplate reader was used to measure the intensity of the color developed. Biotin-antibody (1x) vial was centrifuged and 100-fold dilution was prepared with 10 µl of Biotin-antibody and 990 µl of Biotin-antibody Diluent. HRP- Avidin (1x) vial was centrifuged and 100-fold dilution was prepared 10 µl of HRP- Avidin and 990 µl of HRP- Avidin diluent. Wash buffer was heated up to the room temperature with gentle mixing and 20 ml of Wash Buffer Concentrate (25 x) was diluted with distilled water and 500 ml of Wash Buffer (1 x) was prepared. Standard vial was centrifuged at 6000-10000rpm for 30 seconds and added with gentle agitation to 1 ml of sample diluent which resulted in a solution of 500 pg/ml. 250 µl of sample diluent was added (S0-S6) into each tub. The undiluted Standard was used as the high standard (500 pg/ml) and sample diluent as zero standard (0 pg/ml)

Elisa Procedure

After preparing the reagents, working standards and samples, 100µl of standard and sample were dispensed into each well. An adhesive strip was used to cover the well and this was incubated for 2 hours at 37°C. The solution was aspirated from the wells, 100µl of Biotin-antibody was introduced again and new adhesive strip was used to cover it. This assembly was incubated for 1 hour at 37°C and the whole process repeated twice.

Wash Buffer (200µl) was used to fill the wells and was let to stand for 2 minutes. HRP-Avidin (100µl) was later introduced to each well, and closed with microtiter plate and fresh adhesive strip which was incubated for 1 hour at 37°C. After repeating the aspiration process five times, 90µl of TMB Substrate was introduced and incubation continued. Stop Solution (50µl) was put into the wells and resulting optical density was noted by a microplate reader.

Statistical Analysis

Mean and Standard Deviations (SD) were calculated for all the parameters for all the study groups. (Table 1) The comparison of control and test groups with amount of Cathepsin K at different time intervals was done by Friedman's Test. (Table 2). The comparison of Cathepsin K at different time intervals within the control group and test group was done by Wilcoxon signed rank test. P-value more than 0.05 was considered as statistically significant.

Table 1: Comparison of control and test groups with amount of cathepsin k at different time intervals by Wilcoxon Signed ranked test

Time	Sides	N	Mean	SD	Median	Z	P-Value
0 Hrs	Test	8	144.88	111.33	95.00	-0.140	0.89
	Control	8	142.99	133.26	125.05		
24 Hrs	TT 24 hrs	8	51.59	44.84	27.26	-0.338	0.74
	CT 24 hrs	8	52.39	49.48	30.08		
7th Day	TT 7th day	8	365.28	791.30	96.87	-1.540	0.12
	CT 7th day	8	281.25	662.11	20.10		
30th Day	TT 30th day	8	46.34	35.26	40.69	-1.363	0.17
	CT 30th day	8	91.28	106.65	37.83		

Table 2: Comparison of cathepsin K levels in GCF between test & control sides using Friedman's test

Side	Time	N	Mean	SD	Mean Rank	Min	Max	2Value	P-Value
Test	0 Hr	8	144.88	111.33	3.00	51.3	368.4	2.550	0.46
	24 Hrs	8	51.59	44.84	2.38	8.4	117.8		
	7th Day	8	365.28	791.30	2.63	4.0	2313.8		
	30th Day	8	46.34	35.26	2.00	9.2	94.5		
Control	0 Hr	8	142.99	133.26	3.25	11.0	358.3	4.050	0.26
	24 Hrs	8	52.39	49.48	2.50	8.1	167.1		
	7th Day	8	281.25	662.11	2.13	10.6	1915.0		
	30th Day	8	91.28	106.65	2.13	10.8	283.7		

Result

The results derived from the test side (TT) showed a reduction of GCF levels of Cathepsin K from 24 hour after the application of retraction force, followed by an increased level of Cathepsin K at 7th day (168 hrs) and again decreased GCF levels of Cathepsin K at 30th day.

In the control side (CT), the results showed decreased level of Cathepsin K values in GCF after 24 hours application of retraction force, followed by increased level at 1 week (168 hours), then again decrease level of Cathepsin K at 30th day which was similar to the GCF levels of Cathepsin K in TT.

Another important finding seen was on the 30th day, where Cathepsin K levels were considerably higher on the control side in comparison to the test side.

Discussion

GCF contains a rich array of cellular and biochemical factors indicating the metabolic status of the periodontium. Previous studies have demonstrated that there was difference in permeability when comparing oral epithelia and gingival pocket epithelium.[7, 8] The exchange of fluid across the gingival crevice epithelium was debated as a physiological or pathological process. Many studies have shown that the volume of GCF increased markedly following mechanical stimulation of the gingiva, pathological inflammation or even after systemic introduction of histamine. [9]

Thus it was concluded that any irritation, either chemical or mechanical, leads to the synthesis of GCF.

Brill postulated the beneficial effects and the protective mechanism of GCF in the crevicular region. [10] Cathepsins (Ancient Greek kata- "down" and hepsein - "boil") are proteases recognized in all organisms and approximately twelve different Cathepsins have been identified based on their composition and the proteins they cleave. Cathepsins have an important role in bone resorption controlling cellular turnover.[11]

In the present study GCF was examined by ELISA method to measure the level of Cathepsin K. Another method of detecting and assessing the activity of Cathepsin K is by using a fluoro-substrate, but is unreliable in specificity and sensitivity unlike ELISA which has high sensitivity. Western blot technique was used by Mogi M. et al. to detect RANKL and/or Cathepsin K in GCF but were unable to detect biomarkers from the GCF. Therefore, the ELISA has the ability to detect as well as assess Cathepsin K levels with both specificity and sensitivity.[12]

Results of this study show significant differences when comparing the control and the treated teeth for Cathepsin K, wherein the average values for the treated site significantly greater than the control sites. Cathepsin K was increased in the GCF at 7th day after tooth movement on Friedman's test. At the test side, where laceback was used to retract the canine, the values of secreted Cathepsin K were greater than the control sides at 7th day.

The interpretation of this study indicated that Cathepsin K is involved in very complex cascading chain of events that regulate aseptic inflammation as induced by orthodontic forces. Cathepsin K is a proteolytic enzyme found in the acute phase of inflammatory condition produced by paradental cells and gives as a reasonable explanation about the rapid increase of Cathepsin K in GCF at 7th day.

Another important finding seen was, on the 30th day, where Cathepsin K levels were significantly higher on the control side when compared to the test side. Cathepsin K is expressed and secreted by osteoclasts, and hence it was derived that increase in Cathepsin K levels in the GCF indirectly reflects the formation of osteoclasts. The expression of Cathepsin K could be in response to

inflammatory mediators such as prostaglandins and interleukins.

Study done by Saftig et al. on rats suggested that Cathepsin K was secreted only in odontoclasts and osteoclasts.[13] Another experimental study showed that Cathepsin K levels significantly increased in irradiated group compared with non-irradiated group in the initial phase of treatment.[14] Bonafe et al. did a study and found that novel cysteine protease is highly resorption process during orthodontic tooth movement, despite the magnitude of force remaining constant.[15]

According to all the above mentioned studies, the level of Cathepsin K can be reliably measured during the acute phase of treatment and beyond that the values are unreliable. Hence, the increase in the Cathepsin K levels on control side measured on 30th day in our study can be considered as unreliable since it has been measured after the acute phase of treatment.

These findings suggest that Cathepsin K is an important component in orthodontically mediated Osteoclastic bone resorption induced by mechanical stimulation of periodontal tissues that these mediators can be detected non-invasively in the GCF. However, well designed experimental studies are essential to evaluate their clinical efficiency. Future studies are required further clarify the exact role of Cathepsin K in this cascading complex interaction of the molecules in the GCF during orthodontic treatment.

Conclusion

The orthodontic displacement of a tooth is the result of a mechanical stimulus, which in turn may trigger the cascade of biological events associated with bone remodeling. This leads to the production and activation of various biomolecules like growth factors, cytokines and proteases which are ultimately responsible for initiating bone remodeling and subsequent tooth movement. Any interference with the release of these neurotransmitters and enzymes could delay or hamper orthodontically induced tooth movement.

The result of this study suggest that the amount of Cathepsin K in the GCF increased by orthodontic tooth movement during initial phase of tooth movement which may be involved in extracellular matrix degradation.

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Conflict of Interest: None.

References

1. Griffiths GS. Formation, collection and significance of gingival crevice fluid. *Periodontology* 2000. 2003 1;31(1):32-42.
2. Serra E, Perinetti G. Lactate dehydrogenase activity in gingival crevicular fluid during orthodontic treatment. *Am J Orthod Dentofacial Orthop* 2003;31;124(2):206-11.
3. Krishnan V, Davidovitch ZE. Cellular, molecular, and tissue-level reactions to orthodontic force. *Am J Orthod Dentofacial Orthop* 2006;30;129(4):469-e1.
4. Bergmann M, Fruton JS. Regarding the general nature of catheptic enzymes. Science. 1936 Jul 24;84(2169):89-90.
5. Shi G P et al Molecular cloning of human cathepsin O, a novel endoproteinase and homologue of rabbit OC2. FEBS letters. 1995 Jan 3;357(2):129-34.
6. Drake FH. Cathepsin K, but not cathepsins B, L, or S, is abundantly expressed in human osteoclasts. *J Biol Chem* 1996;24;271(21):12511-6.
7. Rhee SH, Kang J, Nahm DS. Cystatins and cathepsin B during orthodontic tooth movement. *Am J Orthod Dentofacial Orthop* 2009;31;135(1):99-105.
8. Brill N, Björn H. Passage of tissue fluid into human gingival pockets. Acta Odontologica Scandinavica. 1959 Jan 1;17(1):11-21.
9. Brill N, Krasse B. Effect of mechanical stimulation on flow of tissue fluid through gingival pocket epithelium. *Acta Odontol Scand* 1959;17:115–30.
10. Brill N. Influence of capillary permeability on flow of tissue fluid into gingival pockets. *Acta Odontol Scand*. 1959; 1;17(1):23-33.
11. Nomura T, Katunuma N. Involvement of cathepsins in the invasion, metastasis and proliferation of cancer cells. *J Med Invest*. 2005;52(1, 2):1-9.
12. Mogi M, Otogoto J. Expression of cathepsin-K in gingival crevicular fluid of patients with periodontitis. *Arch Oral Biol* 2007:52: 894–8.
13. Saftig P, Hunziker E, Wehmeyer O, Jones S, Boyde A, Rommerskirch W et al. Impaired osteoclastic bone resorption leads to osteopetrosis in cathepsin-K-deficient mice. Proceedings of the National Academy of Sciences. 1998 Nov 10;95(23):13453-8.
14. Yamaguchi M, Hayashi M, Fujita S, Yoshida T, Utsunomiya T, Yamamoto H, Kasai K. Low-energy laser irradiation facilitates the velocity of tooth movement and the expressions of matrix metalloproteinase-9, cathepsin K, and alpha (v) beta (3) integrin in rats. *European J Orthod* 2010;16;32(2):131-9.
15. Zainal Ariffin SH, Yamamoto Z, Abidin Z, Megat Abdul Wahab R, Zainal Ariffin Z. Cellular and molecular changes in orthodontic tooth movement. *Scientific World J* 2011;11:1788-803.

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