EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 7
Issue: 2
433-436
Year: 2020

# ASSESSMENT OF CATHEPSIN K BIOMARKER IN GINGIVAL CREVICULAR FLUID DURING ORTHODONTIC TREATMENT — A CLINICAL STUDY

**Prerna Suri***

BDS, MDS (ORTHODONTICS) Private Practitioner and Consultant Orthodontist Mumbai, India.

***Corresponding Author: Prerna Suri**
BDS, MDS (ORTHODONTICS) Private Practitioner and Consultant Orthodontist Mumbai, India.

**Article Received on 20/12/2019** **Article Revised on 10/01/2020** **Article Accepted on 31/01/2020**

## 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:*** Sixteen 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 left canine which was used as Test Tooth (TT) and maxillary right canine used as Control Tooth (CT) with no lace back. 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 conclude that the amount of Cathepsin K in GCF that increased during the early period of orthodontic tooth movement may indicate a significant role during initial tooth movement.

**KEYWORDS:** Cathepsin K, GCF.

## INTRODUCTION

Gingival crevicular fluid (GCF) is a biological exudate and quantification of its constituents is a current method to identify specific biomarkers with reasonable sensitivity for several biological events. Studies are being performed to evaluate whether the GCF biomarkers in growing subjects reflect both the stages of individual skeletal maturation and the local tissue remodeling triggered by orthodontic force. Present evidence is still little regarding whether and which GCF biomarkers are correlated with the growth phase (mainly pubertal growth spurt), while huge investigations have been reported on several GCF biomarkers (for inflammation, tissue damage, bone deposition and resorption, and other biological processes) in relation to the orthodontic tooth movement. In spite of these investigations, the clinical applicability of the method is still limited with further data needed to reach a full diagnostic utility of specific GCF biomarkers in orthodontics. Future studies are warranted to elucidate the role of main GCF biomarkers and how they can be used to enhance functional treatment, optimize orthodontic force intensity, or prevent major tissue damage consequent to orthodontic treatment.[1]

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. 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. Assessment of these GCF biomarkers is clinically significant because it may lead to better understanding of mechanical stress resulting in expression of some chemical biomarkers which are essential for some desirable effects like controlled tooth movement, so as to plan for 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.[2-4]

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.[5]

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 wellknown marker of osteoclast activity. There are studies which show increased level of Cathepsin K in GCF of patients with periodontitis. 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

16 patients were selected for the study between 12 to 24 years needing orthodontic treatment for Angle's Class I bimaxillary protrusion requiring four first premolar extractions as a treatment plan, 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 lace back were given using 0.010 SS ligature wire only on test tooth side with approximate force values of 200cN as measured on a Dontrix gauge.

GCF samples were collected 1 hour before (baseline), and at 1st day (24 hours), 7th day (168 hours), and 1 month (30 days) after the placement of the orthodontic appliance on maxillary left canines (TT) for test side and maxillary right canines (CT) control side in all individuals.

**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. 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 which uses the solid phase enzyme immunoassay technique was used for the quantification of Cathepsin K values. 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 density developed.

**Statistical Analysis :**The results were tabulated to measure the mean and Standard Deviations (SD) which were calculated for all the parameters for all individuals of the study and control groups. The comparison of Cathepsin K at different time intervals within the control group and test group was done by ANOVA test. P-value less than 0.05 was considered as statistically significant. (Table 1).

**Table 1: Comparison of control and test sides with amount of Cathepsin k at different time intervals by ANOVA test.**

| Time | Side | Mean± SD | p-Value |
|---|---|---|---|
| **Baseline** | Test | 156.46±10.26 | 0.76 |
| | Control | 149.34±12.06 | |
| **24 hrs** | Test | 55.61±2.05 | 0.68 |
| | Control | 128.61±3.16 | |
| **7th Day** | Test | 408.88±11.26 | 0.04* |
| | Control | 196.88±9.8 | |
| **30th Day** | Test | 162.26± 7.05 | 0.19 |
| | Control | 103.01±8.04 | |

## 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 a significant increased level of Cathepsin K at 7th day (168 hrs)as compared with the control tooth GCF Cthepsin K levels and again decreased GCF levels of Cathepsin K at 30th day. In the control side (CT), the results showed insignificant changes in the levels of Cathepsin K values in GCF after 24 hours and one week of application of retraction force. Control tooth side on 30th day was not

changed and was similar to the GCF levels of Cathepsin K in Test tooth side which were near to the base line level.

## 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. The exchange of fluid across the gingival crevice epithelium was debated as a physiological or pathological process.[7] 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.[8,9]

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 test side significantly greater than the control side. 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.[13]

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 which exhibits proteolytic properties primarily of Type 1 collagen present in bone. 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 which can be detected noninvasively 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.[16,17]

## CONCLUSION

Orthodontic tooth movement is achieved by the remodeling of periodontal ligament and alveolar bone in response to the mechanical loading and is believed to be mediated by several proinflammatory mediaters, such as cytokines and protease like enzymes. These mediators are known to trigger the process by a cascade of cellular events for initiating bone remodeling and subsequent tooth movement. The result of this study suggest that the amount of Cathepsin K in the GCF increased during initial phase of teeth retraction which may be involved in degradation of extracellular matrix of bone and thus playing a regulatory role in orthodontic force induced alveolar bone remodeling. The periodontium soon stabilizes at a new physiological homeostatsis with the downregulation of early phase proinflammatory cytokines.

## REFERENCES

1. Meikle MC. The tissue, cellular, and molecular regulation of orthodontic tooth movement: 100 Years after Carl Sandstedt. *European Journal of Orthodontics*, 2006; 28(3): 221–240.
2. Davidovitch Z, Krishnan V. Role of basic biological sciences in clinical orthodontics: a case series.

*American Journal of Orthodontics and Dentofacial Orthopedics*, 2009; 135(2): 222–231.
3. Iwasaki LR, Haack JE, Nickel JC, Reinhardt RA, Petro TM. Human interleukin-1*β* and interleukin-1 receptor antagonist secretion and velocity of tooth movement. *Archives of Oral Biology*, 2001; 46(2): 185–189.
4. Salla JT, Taddei SRDA, Queiroz-Junior CM, Andrade Junior I, Teixeira MM, Silva TA. The effect of IL-1 receptor antagonist on orthodontic tooth movement in mice. *Archives of Oral Biology*, 2012; 57(5): 519–524.
5. Zainal Ariffin SH, Yamamoto Z, Zainol Abidin LZ, Megat Abdul Wahab R, Zainal Ariffin Z. Cellular and molecular changes in orthodontic tooth movement. *TheScientificWorldJournal*, 2011; 16: 761768.
6. Kook S-H, Jang Y-S, Lee J-C. Human periodontal ligament fibroblasts stimulate osteoclastogenesis in response to compression force through TNF-*α*-mediated activation of CD4+ T cells. *Journal of Cellular Biochemistry*, 2011; 112(10): 2891–2901.
7. Gostner JM, Becker K, Fuchs D, Sucher R. Redox regulation of the immune responce. *Redox Report*, 2013; 18: 88–94.
8. Padgett LE, Broniowska KA, Hansen PA, Corbett JA, Tse HM. The role of reactive oxygen species and proinflammatory cytokines in type 1 diabetes pathogenesis. *Annals of the New York Academy of Sciences*, 2013; 1281: 16–35.
9. Çanakçi CF, Çanakçi V, Tatar A, et al. Increased salivary level of 8-hydroxydeoxyguanosine is a marker of premature oxidative mitochondrial DNA damage in gingival tissue of patients with periodontitis. *Archivum Immunologiae et Therapiae Experimentalis*, 2009; 57(3): 205–211.
10. Canakci CF, Cicek Y, Yildirim A, Sezer U, Canakci V. Increased levels of 8-hydroxydeoxyguanosine and malondialdehyde and its relationship with antioxidant enzymes in saliva of periodontitis patients. *European Journal of Dentistry*, 2009; 3: 100–106.
11. Ren Y, Hazemeijer H, de Haan B, Qu N, de Vos P. Cytokine profiles in crevicular fluid during orthodontic tooth movement of short and long durations. *Journal of Periodontology*, 2007; 78(3): 453–458.
12. Bletsa A, Berggreen E, Brudvik P. Interleukin-1*α* and tumor necrosis factor-*α* expression during the early phases of orthodontic tooth movement in rats. *European Journal of Oral Sciences*, 2006; 114(5): 423–429.
13. Alhashimi N, Frithiof L, Brudvik P, Bakhiet M. Orthodontic tooth movement and de novo synthesis of proinflammatory cytokines. *American Journal of Orthodontics and Dentofacial Orthopedics*. 2001; 119(3): 307–312.
14. Sezer U, Çiçek Y, Çanakçı CF. Increased salivary levels of 8-hydoxdeoxguanosine may be marker for disease activity for periodontitis. *Disease Markers*, 2012; 32: 165–172.
15. Dahiya P, Kamal R, Gupta R, Bhardwaj R, Chaudhary K, Kaur S. Reactive oxygen species in periodontitis. *Journal of Indian Society of Periodontology*, 2013; 17: 411–416.
16. Bertoli DR, Nedwin GE, Bringman TS, Smith DD, Mundy GR. Stimulation of bone resorption and inhibition of bone formation in vitro by human tumour necrosis factors. *Nature*, 1986; 319(6053): 516–518.
17. Andrade I, Jr., Taddei SR, Souza PEA. Inflammation and tooth movement: the role of cytokines, chemokines, and growth factors. *Seminars in Orthodontics*, 2012; 18(4): 257–270.