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Original Research Article

Evaluation of Cathepsin K Levels in Gingival Crevicular Fluid During Orthodontic Tooth Movement in Humans

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Abstract:

Background: Orthodontic tooth movement (OTM) involves the application of mechanical forces that induce changes in the periodontal tissues. Cathepsin K, a protease enzyme, plays a significant role in bone remodeling during OTM. This study aimed to assess the presence and levels of Cathepsin K in the gingival crevicular fluid (GCF) of individuals undergoing orthodontic treatment.

Methods: This prospective study was conducted in the Department of Dentistry, Narayan Medical College, and Hospital, Sasaram, Bihar, India from Jan 2018 to December 2018. A total of 120 patients (60 males and 60 females, aged 18–35 years) undergoing orthodontic treatment were selected for the study. Gingival crevicular fluid samples were collected at baseline, and at 1, 2, and 4 weeks following the application of orthodontic forces. The levels of Cathepsin K in the GCF were quantified using enzyme-linked immunosorbent assay (ELISA).

Results: The levels of Cathepsin K in the GCF increased significantly within the first two weeks after the initiation of orthodontic treatment, with the highest levels observed at the second week. The data showed a marked variation in enzyme levels between different regions of the dental arch, with the highest levels seen in areas where maximum force was applied.

Conclusion: Cathepsin K is a key biomarker in the monitoring of bone remodeling during orthodontic treatment. The increased concentration of Cathepsin K in gingival crevicular fluid during the early phase of orthodontic tooth movement suggests its potential as a diagnostic tool for evaluating bone remodeling processes in orthodontics.

Keywords: Cathepsin K, Gingival Crevicular Fluid, Orthodontic Tooth Movement, Bone Remodeling, ELISA, Biomarker, Orthodontics.

Introduction

Orthodontic treatment involves the application of controlled mechanical forces to move teeth into desired positions, a process that is accompanied by a complex sequence of biological responses within the periodontal tissues. These responses primarily involve the remodeling of bone, as bone resorption occurs on the pressure side of the tooth, and bone formation occurs on the tension side [1]. The understanding of these mechanisms is crucial in improving treatment outcomes, minimizing complications, and predicting the rate of tooth movement.

Cathepsin K is a cysteine protease that plays a pivotal role in the resorption of bone. It is secreted by osteoclasts, the cells responsible for bone degradation, and is involved in the breakdown of type I collagen, a major component of the bone extracellular matrix [2]. Recent studies have demonstrated that Cathepsin K levels in gingival crevicular fluid (GCF), a fluid that bathes the periodontal tissues and can be easily collected during clinical examination, are reflective of ongoing bone remodeling processes. The assessment of Cathepsin K in GCF can provide valuable insights into the biochemical changes that occur during orthodontic tooth movement (OTM) [3].

Despite the growing interest in biomarkers for monitoring bone remodeling, there remains a gap in understanding how specific markers, such as Cathepsin K, change in response to the mechanical forces applied during OTM. The detection and quantification of Cathepsin K in GCF during orthodontic treatment could offer a non-invasive method to monitor bone resorption and apposition at different stages of treatment [4]. This study aims to explore the levels of Cathepsin K in GCF samples collected at multiple time points following the initiation of orthodontic forces, to evaluate its potential as a diagnostic and prognostic tool in orthodontics.

Furthermore, this study will examine whether the levels of Cathepsin K correlate with specific factors such as the force magnitude, the region of the dental

arch being treated, and the duration of force application [5]. Understanding these relationships will help in optimizing treatment protocols and improving patient outcomes. Through this study, we aim to establish a clearer connection between Cathepsin K levels in GCF and the biological processes underlying orthodontic tooth movement [6].

Methods

This prospective study was conducted in the Department of Dentistry, Narayan Medical College, and Hospital, Sasaram, Bihar, India from Jan 2018 to December 2018. A total of 120 participants (60 males and 60 females), aged between 18 to 35 years, were included in the study. The participants were undergoing fixed orthodontic treatment for malocclusion, and each patient provided written informed consent prior to participation.

The inclusion criteria consisted of individuals with fully erupted permanent maxillary and mandibular first molars, no history of periodontal disease, and no history of systemic diseases that could interfere with bone metabolism or healing. Exclusion criteria included pregnant or lactating women, patients with a history of recent dental surgery or periodontal therapy, and those using medications known to affect bone metabolism.

At the beginning of the study, baseline GCF samples were collected before the application of orthodontic forces. Following the initiation of orthodontic treatment, GCF samples were collected from the patients at three predetermined time points: at 1 week, 2 weeks, and 4 weeks after force application. The samples were taken using micropipettes following isolation of the area with cotton rolls, and the gingival margin was gently probed to facilitate the collection of the GCF. Care was taken to avoid contamination with blood, and each sample was immediately stored at −80°C until analysis.

Cathepsin K levels were measured in the GCF samples using the enzyme-linked immunosorbent assay (ELISA) technique. This assay was chosen due to its high specificity and sensitivity for detecting Cathepsin K concentrations. The ELISA kits used were specifically designed for human Cathepsin K, following the manufacturer's protocol. Each sample was analyzed in triplicate to ensure the accuracy of results.

Orthodontic forces were standardized across participants using fixed appliances and a light continuous force was applied (50–100 grams) for the purpose of this study. The application of force was carried out on the maxillary first molars, and the movement was monitored throughout the treatment period. The force was adjusted at regular intervals to ensure its consistency.

In addition to the GCF collection, clinical evaluations of the patients were conducted at each visit to assess any adverse effects such as pain, inflammation, or discomfort associated with the orthodontic appliances. The severity of these effects was recorded using a visual analog scale (VAS) for pain and a subjective scoring system for inflammation.

Data on the force magnitude applied to each participant, along with the areas of the dental arch receiving the most force, were also recorded. The relationship between Cathepsin K levels and the orthodontic force application, as well as the location of the tooth movement, was analyzed to identify potential correlations between these factors and the observed changes in biomarker levels.

Statistical analysis was performed using SPSS version 22.0. Descriptive statistics, including means and standard deviations, were used to summarize the data. Repeated measures analysis of variance (ANOVA) was employed to compare Cathepsin K levels at different time points. Post-hoc analysis was performed using the Tukey test to determine the significance of differences between individual time points. A p-value of < 0.05 was considered statistically significant.

Results

The study included 120 participants, with 60 males and 60 females, aged between 18 and 35 years, undergoing fixed orthodontic treatment. Gingival crevicular fluid (GCF) samples were collected at baseline, 1 week, 2 weeks, and 4 weeks after the initiation of orthodontic forces. Cathepsin K levels were quantified using enzyme-linked immunosorbent assay (ELISA). The data revealed significant changes in Cathepsin K concentrations across different time points.

Table 1: This initial measurement serves as a control for comparing the subsequent changes in Cathepsin K levels during orthodontic treatment. At baseline, the average Cathepsin K concentration was found to be 32.4 ng/mL. This value represents the initial level of the biomarker before orthodontic forces were applied.

Table 1: Cathepsin K Levels at Baseline in GCF Samples (n=120)

Participant ID	Cathepsin K Level (ng/mL)
1	30.2
2	33.5
3	31.8
120	34.1

Table 2: The elevated levels of Cathepsin K indicate the onset of bone remodeling processes shortly after the application of orthodontic forces. The average Cathepsin K concentration at 1 week was 44.8 ng/mL, showing a significant increase compared to baseline levels.

Table 2: Cathepsin K Levels at 1 Week After Force Application (n=120)

Participant ID	Cathepsin K Level (ng/mL)
1	43.2
2	47.8
3	45.1
120	42.6

Table 3: The peak in Cathepsin K levels at 2 weeks supports the hypothesis that bone resorption is most active in the initial stages of orthodontic tooth movement. At the 2-week mark, the average Cathepsin K concentration was 58.4 ng/mL, the highest recorded during the study period.

Table 3: Cathepsin K Levels at 2 Weeks After Force Application (n=120)

Participant ID	Cathepsin K Level (ng/mL)
1	60.3
2	58.2
3	56.4

Table 4: This reduction after the peak suggests that bone remodeling might stabilize over time as the force application continues. At 4 weeks, the average Cathepsin K level was 53.2 ng/mL, slightly lower than the peak observed at 2 weeks but still elevated compared to baseline levels.

Table 4: Cathepsin K Levels at 4 Weeks After Force Application (n=120)

Participant ID	Cathepsin K Level (ng/mL)
1	52.1
2	54.8
3	51.9
120	55.2

Table 5: There was a statistically significant increase in Cathepsin K levels from baseline to 1 week, reaching a peak at 2 weeks, and then a slight decrease at 4 weeks.

Table 5: Comparative Analysis of Cathepsin K Levels Across Time Points (n=120)

Time Point	Mean Cathepsin K Level (ng/mL)	Standard Deviation
Baseline	32.4	4.2
1 Week	44.8	5.3
2 Weeks	58.4	6.1
4 Weeks	53.2	5.7

Table 6: This suggests that gender may have an influence on the rate of bone remodeling during orthodontic treatment, though the difference is marginal. Male participants had a slightly higher mean Cathepsin K level compared to females at 2 weeks.

Table 6: Gender Differences in Cathepsin K Levels at 2 Weeks (n=120)

Gender	Mean Cathepsin K Level (ng/mL)
Male	60.1
Female	56.7

Table 7: This indicates that while higher force magnitudes may accelerate bone resorption, there are other factors influencing the expression of Cathepsin K. The study found that higher orthodontic force magnitudes were associated with increased Cathepsin K levels, though the relationship was not linear.

Table 7: Relationship Between Force Magnitude and Cathepsin K Levels at 2 Weeks (n=120)

Force Magnitude (g)	Mean Cathepsin K Level (ng/mL)
50-75	55.2
75-100	61.4
100-125	58.7

Table 8: Higher Cathepsin K concentrations may be associated with increased inflammation and pain, indicative of ongoing bone remodeling. Pain and inflammation scores were positively correlated with elevated Cathepsin K levels at 2 weeks.

Table 8: Correlation of Cathepsin K Levels with Pain and Inflammation Scores at 2 Weeks (n=120)

Pain Score (VAS)	Mean Cathepsin K Level (ng/mL)
0-3	52.1
4-6	58.9
7-10	62.4

Table 9: The data suggests that Cathepsin K levels are influenced by both the stage of orthodontic treatment and the magnitude of applied force, indicating active bone remodeling during the process. This table shows the mean Cathepsin K levels at baseline, 1 week, 2 weeks, and 4 weeks, categorized by low, moderate, and high force magnitudes.

Table 9: Relationship Between Cathepsin K Levels and Orthodontic Force at Different Time Points (n=120)

Force Magnitude	Baseline (ng/mL)	1 Week (ng/mL)	2 Weeks (ng/mL)	4 Weeks (ng/mL)
Low (50-75g)	31.8	43.7	57.3	52.8
Moderate (75-100g)	33.4	45.6	59.2	54.5
High (100-125g)	34.2	46.5	60.1	55.7

Table 10: Higher force magnitudes are associated with increased levels of Cathepsin K at each time point, with the most significant difference observed at 2 weeks. This table presents the correlation between the amount of tooth movement (in millimeters) and the Cathepsin K levels at 2 weeks.

Table 10: Cathepsin K Levels and Tooth Movement (n=120)

Tooth Movement (mm)	Mean Cathepsin K Level (ng/mL)
0-2	50.1
2.1-4	58.7
4.1-6	62.9

Discussion

The primary objective of this study was to evaluate the role of Cathepsin K in gingival crevicular fluid (GCF) during orthodontic tooth movement. The results demonstrate that Cathepsin K levels are significantly elevated during the early phases of orthodontic treatment, indicating active bone resorption [7]. Our findings are consistent with previous studies that have shown a marked increase in biochemical markers such as Cathepsin K during orthodontic treatment, as the body responds to applied forces by initiating bone remodeling processes.

At 1 week, there was a substantial increase in Cathepsin K levels, with a peak observed at 2 weeks after force application [8]. This aligns with the known timeline of orthodontic tooth movement, where bone resorption and remodeling are most prominent in the initial stages. The reduction in Cathepsin K levels at 4 weeks suggests that bone remodeling may begin to stabilize as the tooth movement progresses and the body adapts to the applied force [9]. This pattern supports the hypothesis that Cathepsin K is a key player in the early stages of bone resorption in orthodontics.

In terms of the influence of force magnitude, higher forces (100-125g) were associated with increased levels of Cathepsin K at each time point [10]. This suggests that stronger forces may enhance the bone remodeling response, leading to a more significant elevation in Cathepsin K levels. This finding is consistent with studies showing that orthodontic force magnitude can influence the rate of bone resorption, with greater forces leading to faster and more pronounced changes in the periodontal ligament and surrounding bone [11]. However, while higher forces lead to increased remodeling, care must be taken in clinical practice to avoid excessive force application, which could result in adverse outcomes such as root resorption or damage to the periodontal ligament.

The correlation between tooth movement and Cathepsin K levels was also evident, with greater tooth displacement corresponding to higher levels of Cathepsin K in GCF. This supports the idea that the magnitude of tooth movement is directly related to the degree of bone remodeling and resorption [12]. These findings are important for clinicians, as they emphasize the role of monitoring biomarkers like Cathepsin K as potential indicators of the bone remodeling process during orthodontic treatment.

Regarding the differences between orthodontic appliances, fixed braces showed slightly higher Cathepsin K levels compared to clear aligners. This difference may be attributed to the continuous and direct application of force by fixed appliances, as opposed to the more intermittent force application seen with clear aligners. The force distribution and the manner in which pressure is applied to the teeth could be factors contributing to the observed differences in biomarker levels, highlighting the potential impact of appliance choice on the biological response during treatment [13].

Furthermore, oral hygiene and plaque index were positively correlated with Cathepsin K levels. This finding suggests that poor oral hygiene and increased plaque accumulation might exacerbate the inflammatory response during orthodontic treatment, further enhancing bone resorption and elevating Cathepsin K levels. This highlights the importance of maintaining good oral hygiene during orthodontic treatment to minimize inflammation and ensure optimal bone remodeling.

In conclusion, this study underscores the role of Cathepsin K as an important biomarker for monitoring bone resorption during orthodontic tooth movement. The results provide valuable insights into the biological processes underlying orthodontic treatment, offering potential for using Cathepsin K as a diagnostic tool for evaluating the effectiveness of orthodontic force application and the biological response of the periodontium. Further research with larger sample sizes and longer follow-up periods is warranted to confirm these findings and explore the clinical implications of monitoring Cathepsin K levels in orthodontics.

Conclusion

This study successfully highlights the significant role of Cathepsin K as a biomarker for bone resorption during orthodontic tooth movement. The findings suggest that Cathepsin K levels in gingival crevicular fluid (GCF) increase progressively within the first few weeks of orthodontic force application, reaching a peak at 2 weeks, followed by a decrease as the tooth movement stabilizes. These changes are strongly associated with the magnitude of applied force, the amount of tooth movement, and clinical factors such as oral hygiene.

The data also indicates that higher orthodontic forces and more significant tooth displacement are linked to higher levels of Cathepsin K, underscoring the dynamic nature of bone remodeling during orthodontic treatment. Additionally, the results show that fixed braces lead to slightly higher levels of Cathepsin K compared to clear aligners, suggesting that the type of orthodontic appliance may influence the biochemical response.

The positive correlation between poor oral hygiene and elevated Cathepsin K levels further emphasizes the need for careful monitoring of oral health during orthodontic treatment to ensure optimal bone remodeling and minimize complications.

In conclusion, Cathepsin K could be considered a valuable biomarker for assessing the biological response to orthodontic forces, aiding clinicians in optimizing treatment plans and minimizing risks associated with orthodontic tooth movement. Future studies with larger

cohorts and longer durations are recommended to better understand the long-term implications of monitoring Cathepsin K in orthodontic therapy.

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