Comparative evaluation of pentraxin 3 levels in GCF during canine retraction with active tieback and NiTi coil spring: An *in vivo* study

Pratik Patel, Ravi Shanthraj, A Bhagyalakshmi, Nekta Garg and Anisha Vallakati

ABSTRACT

Objectives: To compare the levels of pentraxin 3 (PTX-3) in gingival crevicular fluid (GCF) in patients undergoing orthodontic canine retraction with active tieback and nickel titanium (NiTi) coil spring.
Materials and Methods: Fifteen patients of the age group 15–25 years with first premolar extraction undergoing canine retraction were selected. One month after placement of 0.019” × 0.025” stainless steel wire, canine retraction was started with active tieback (150 g force) on upper right quadrant and NiTi coil spring (150 g force) on upper left quadrant. GCF samples were collected 1 h before commencement of canine retraction and thereafter at intervals of 1 h, 1 day, 1 week, and 2 weeks after application of force. The collected GCF was eluted from the microcapillary pipette in 100 µl phosphate-buffered saline (pH 5–7.2). The samples were analyzed for PTX-3 levels by the ELISA technique.
Results: The mean levels of PTX-3 at 1 h before canine retraction (baseline) was 1.30 ± 0.22 ng/ml and at 1 h 1.66 ± 0.33 ng/ml, 1 day 2.65 ± 0.09 ng/ml, 1 week 1.96 ± 0.15 ng/ml, and 2 weeks 1.37 ± 0.18 ng/ml in active tieback group. The mean levels of PTX-3 at 1 h before canine retraction was 1.32 ± 0.30 ng/ml, and at 1 h 1.71 ± 0.39 ng/ml, 1 day 2.78 ± 0.12 ng/ml, 1 week 2.52 ± 0.18 ng/ml, and 2 weeks 2.12 ± 0.17 ng/ml in NiTi coil spring group. A significant difference of $P < 0.001$ was found in PTX-3 levels in GCF during canine retraction between active tieback and NiTi coil spring at 1 day, 1 week, and 2 weeks.
Conclusion: The results showed that PTX-3 levels increased from 1 h after application of orthodontic force and reached peak at 1 day, followed by a gradual decrease at 1 week and 2 weeks in both active tie back and NiTi coil spring groups.

Key words: Active tieback, gingival crevicular fluid, nickel titanium coil spring, pentraxin 3

INTRODUCTION

Gingival crevicular fluid (GCF) is an exudate that precisely reflects biologic events of the periodontium and is used to detect the levels of certain biomarkers.[1] Expression of biologically active substances such as cytokines,[2] matrix metalloproteinases (MMPs) and their inhibitors,[3] osteoprotegerin,[4] tumor necrosis factor (TNF),[5] neuropeptides,[6] lactate dehydrogenase (LDH),[7] aspartate aminotransferase,[8] and leptin[9] were studied in GCF during orthodontic tooth movement. Pentraxin 3 (PTX-3), also known as TNF-stimulated gene 14, is a 45-kDa glycoprotein with a 202 amino-acids C-terminal pentraxin domain, which is longer than that found in other pentraxins such as C-reactive protein (CRP) and

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serum amyloid P.[10] PTX-3 is a "long" pentraxin produced, especially by fibroblasts, macrophages,[11] and neutrophils.[12] Elevated level of PTX-3 in plasma are seen in severe infections and some diseases.[13,14] PTX-3 is considered as a marker of inflammation.[15]

The closure of extraction space during orthodontic tooth movement can be achieved by two techniques (a) friction (sliding) mechanics and (b) frictionless (loop) mechanics.[16] The ideal force delivery system should meet the following criteria:[17] It should provide optimal tooth moving forces, comfortable for the patient, minimal chairside time, minimal patient cooperation, and economical. The optimal force level for retracting canines has been indicated to be in the range of 150–250 g.[18] The auxiliaries used for space closure in sliding mechanics are coil springs (nickel titanium [NiTi] and stainless steel [SS]), elastic auxiliaries, and magnets.[19] Elastic auxiliaries may be elastics, elastic threads, E-chains, synthetic nonlatex elastic modules, or active tieback.[20] In the daily practice, active tieback is simple, economical, and reliable.[20] NiTi coil springs have been shown to produce a constant force over varying lengths and duration, with no force decay.[21] Active tieback and NiTi coil spring are preferred for extraction space closure. This study was aimed to compare the levels of PTX-3 in GCF in patients undergoing orthodontic canine retraction with active tieback (150 g force) and NiTi coil spring (150 g force).

MATERIALS AND METHODS

Fifteen patients, ranged between 15 and 25 years, who needed canine retraction as a part of fixed orthodontic treatment, were selected. Ethical clearance was obtained from the Institutional Ethical Review Board before conducting the study, which followed the Helsinki guidelines. The procedure was explained in detail to the selected subjects and written informed consent was obtained.

The materials used for the preparation of samples were volumetric microcapillary pipette, polypropylene tubes and 100 μl phosphate-buffered saline. ELISA kit (Boster, Pleasanton, CA, USA) was used to analyze PTX-3 levels in the collected samples. The patients were enrolled after meeting the following inclusion criteria: General good health status, nonsmoking, clinically and radiological healthy periodontal tissues (no gingival bleeding, probing depths 3 mm, and no radiographic evidence of periodontal bone loss), no antibiotic therapy in the past 3 months, no use of anti-inflammatory drugs in the previous 30 days, good oral hygiene, and requiring upper canine retraction with first premolar extraction. Exclusion criteria: Periodontally compromised patients, patients with oral manifestations of disease or a chronic debilitating disease, pregnant, and nursing mothers were all excluded from the study.

In the selected patients 0.022" × 0.028" slot MBT® (Ormco, Orange, CA, USA) preadjusted edge-wise appliance was used. Alignment and leveling of the upper arch were carried out and 1 month after the placement of 0.019" × 0.025" SS wire (Ormco, Orange, CA, USA) canine retraction was started. Split mouth technique was carried out. Active tieback (150 g force) was used on the upper right quadrant [Figure 1a] and NiTi coil spring (American Brace Component and Device, Coimbatore, Tamil Nadu, India) (9 mm length, 150 g force) was used on the upper left quadrant [Figure 1b]. The applied orthodontic force was measured using dontrix gauge (Dentsply, York, PA, USA). GCF samples were collected 1 h prior to the commencement of orthodontic canine retraction and at intervals of 1 h, 1 day, 1 week, and 2 weeks after the commencement of canine retraction. Samples were collected from the distal side of the upper canines. The site was isolated with cotton, an air syringe, and a saliva ejector was used to avoid any salivary contamination. GCF samples were obtained by placing calibrated, volumetric microcapillary pipette (Kimble®, Sigma Aldrich Corporation, Bengaluru, Karnataka, India) of the internal diameter of 1.1 mm with a capacity of 5 μl extracrevicularly over test sites. From each test site, a standardized volume of 5 μl was collected. The absorbed GCF was eluted from the microcapillary pipette in 100 μl phosphate-buffered saline (pH - 7.2). The eluted samples were stored in polypropylene tubes at −20°C prior to analysis. Samples were analyzed with ELISA kit according to manufacturer's instructions. Reading was performed at 450 nm with a correction at 540 nm to reduce optical imperfections on the reading plate (Bio-Rad, iMark Microplate Reader, Hercules, CA, USA).

Statistical Analysis

Included descriptive statistics and repeated measures ANOVA was performed to compare PTX-3 levels in GCF by active tieback and NiTi coil spring at baseline, 1 h, 1 day, 1 week and 2 weeks. Paired-sample *t*-tests were performed to compare the means of two variables for active tieback and NiTi coil spring group. Independent samples *t*-tests were performed to compare PTX-3 levels in GCF between active tie back and NiTi coil spring at same time intervals. All the statistical methods were carried out through the Statistical Package for the Social Sciences version 16.0 (SPSS Inc., Chicago IL, USA), IBM® Corp. (International Business Machines Corporation, Armonk, New York, USA).

RESULTS

The mean levels of PTX-3 at 1 h before canine retraction were 1.30 ± 0.22 ng/ml and at 1 h 1.66 ± 0.33 ng/ml,

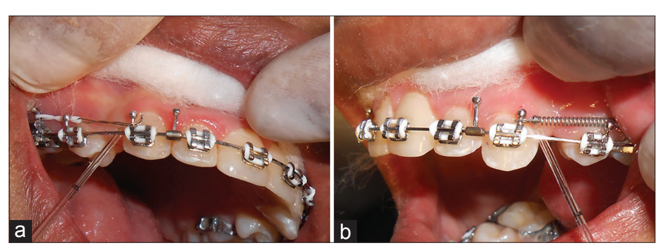

Figure 1: (a) Gingival crevicular fluid sample collection - Active tie back. (b) Gingival crevicular fluid sample collection - nickel titanium coil spring

1 day 2.65 ± 0.09 ng/ml, 1 week 1.96 ± 0.15 ng/ml, and 2 weeks 1.37 ± 0.18 ng/ml in active tieback group. The mean levels of PTX-3 levels at 1 h, before canine retraction were 1.32 ± 0.30 ng/ml and at 1 h 1.71 ± 0.39 ng/ml, 1 day 2.78 ± 0.12 ng/ml, 1 week 2.52 ± 0.18 ng/ml, and 2 weeks 2.12 ± 0.17 ng/ml in NiTi coil spring group [Table 1].

The results obtained with active tieback group showed an increase in GCF levels of PTX-3 from 1 h before the commencement of canine retraction to a maximum at 1 day followed by a gradual decrease reaching nearer to the baseline level at 2 weeks. There was highly statistically significant difference ($P \leq 0.005$) found between mean levels of PTX-3 1 h before canine retraction and after 1 h 1.66 ± 0.33 ng/ml, 1 day 2.65 ± 0.09 ng/ml, 1 week 1.96 ± 0.15 ng/ml, and 2 weeks 1.37 ± 0.18 ng/ml in active tieback group [Table 2].

In orthodontic patients with NiTi coil spring, the results showed an increase in GCF levels of PTX-3 from 1 h after the commencement of orthodontic canine retraction to a maximum at 1 day followed by a gradual decrease at 1 week and 2 weeks. There was highly statistically significant difference ($P \leq 0.005$) found between mean levels of PTX-3 before 1 h of canine retraction 1.32 ± 0.30 ng/ml, and after 1 h 1.71 ± 0.39 ng/ml, 1 day 2.78 ± 0.12 ng/ml, 1 week 2.52 ± 0.18 ng/ml, and 2 weeks 2.12 ± 0.17 ng/ml in NiTi coil spring group [Table 3].

PTX-3 levels were increased more in NiTi coil spring group compared to active tie back group from 1 h to 1 day [Figure 2]. PTX-3 levels were decreased more in active tieback group compared to NiTi coil spring group from 1 day to 2 weeks [Table 4]. PTX-3 levels reached closer to the baseline level within 2 weeks in active tieback group, but the decrease was slower and less in NiTi coil spring group and not reached the baseline level. The findings suggested that there was statistically significant difference in PTX-3 levels in GCF during canine retraction between active tieback and NiTi coil spring groups at 1 day, 1 week, and 2 weeks [Table 4]. On the contrary, there was no statistically

Table 1: Pentraxin 3 levels in gingival crevicular fluid during canine retraction by active tie back and nickel titanium coil spring

Interval	Mean±SD (ng/ml)	
	Active tieback	NiTi coil spring
Baseline	1.30±0.22	1.32±0.30
1 h	1.66±0.33	1.71±0.39
1 day	2.65±0.09	2.78±0.12
1 week	1.96±0.15	2.52±0.18
2 weeks	1.37±0.18	2.12±0.17

SD – Standard deviation; NiTi – Nickel titanium

Table 2: Comparison of pentraxin 3 levels in gingival crevicular fluid by active tie back at different time intervals

Interval	Mean±SD (ng/ml)	*P*
Baseline	1.30±0.22	<0.001
1 h	1.66±0.33	
Baseline	1.30±0.22	<0.001
1 day	2.65±0.09	
Baseline	1.30±0.22	<0.001
1 week	1.96±0.15	
Baseline	1.30±0.22	0.002
2 weeks	1.37±0.18	
1 h	1.66±0.33	<0.001
1 day	2.65±0.09	
1 h	1.66±0.33	<0.001
1 week	1.96±0.15	
1 h	1.66±0.33	<0.001
2 weeks	1.37±0.18	
1 day	2.65±0.09	<0.001
1 week	1.96±0.15	
1 day	2.65±0.09	<0.001
2 weeks	1.37±0.18	
1 week	1.96±0.15	<0.001
2 weeks	1.37±0.18	

Table 3: Comparison of pentraxin 3 levels in gingival crevicular fluid by nickel titanium coil spring at different time intervals

Interval	Mean±SD (ng/ml)	*P*
Baseline	1.32±0.30	<0.001
1 h	1.71±0.39	
Baseline	1.32±0.30	<0.001
1 day	2.78±0.12	
Baseline	1.32±0.30	<0.001
1 week	2.52±0.18	
Baseline	1.32±0.30	<0.001
2 weeks	2.12±0.17	
1 h	1.71±0.39	<0.001
1 day	2.78±0.12	
1 h	1.71±0.39	<0.001
1 week	2.52±0.18	
1 h	1.71±0.39	<0.005
2 weeks	2.12±0.17	
1 day	2.78±0.12	<0.001
1 week	2.52±0.18	
1 day	2.78±0.12	<0.001
2 weeks	2.12±0.17	
1 week	2.52±0.18	<0.001
2 weeks	2.12±0.17	

Table 4: Comparison of pentraxin 3 levels in gingival crevicular fluid by active tie back and nickel titanium coil spring

Interval	Group	Mean±SD (ng/ml)	*P*
Baseline	Active tieback	1.30±0.22	0.805
	NiTi coil spring	1.32±0.30	
1 h	Active tieback	1.66±0.33	0.707
	NiTi coil spring	1.71±0.39	
1 day	Active tieback	2.65±0.09	<0.001
	NiTi coil spring	2.78±0.12	
1 week	Active tieback	1.96±0.15	<0.001
	NiTi coil spring	2.52±0.18	
2 weeks	Active tieback	1.37±0.18	<0.001
	NiTi coil spring	2.12±0.17	

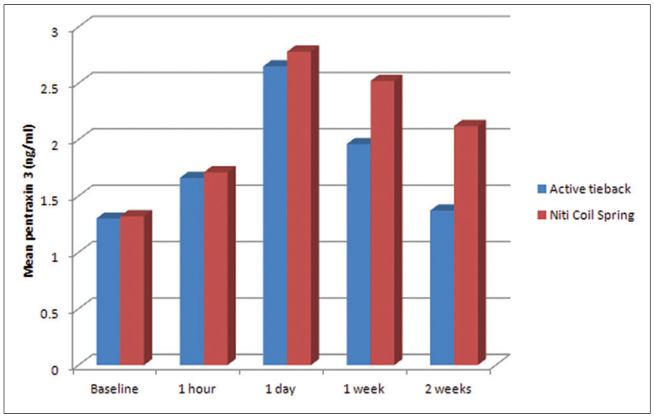

Figure 2: Comparison of pentraxin 3 levels in gingival crevicular fluid by active tieback and nickel titanium coil spring

significant difference in PTX-3 levels at baseline and 1 h between these two groups.

This suggests that the GCF levels of PTX-3 in the periodontium are influenced by orthodontic forces applied by active tieback and NiTi coil spring.

DISCUSSION

PTX-3 is an acute phase protein that is involved in the modulation of the aseptic inflammatory reaction. Therefore, during initial orthodontic treatment, its level rapidly increases and reaches its peak by 24 h, after which it decreases. Thus, it can be a potential early biomarker in orthodontic tooth movement.[10]

In this study, we evaluated PTX-3 levels in GCF during canine retraction by active tieback and NiTi coil spring at different time intervals. Surlin *et al.*[10] showed increased GCF levels of PTX-3 from baseline 1.05 ± 0.67 ng/ml to a maximum at 24 h 2.69 ± 0.64 ng/ml, followed by a decrease in both groups of adult and young patients. PTX-3 seems to be a marker of inflammation better than CRP in dengue.[13] PTX-3 levels in plasma were higher in patients with sepsis than in healthy people.[22] PTX-3 concentration was found to be increased in GCF and plasma in periodontal disease and is considered as an inflammatory marker.[23] In our study, PTX-3 levels increased from baseline to 1 h and reached a peak level at 1 day followed by decrease at 1 week and 2 weeks in both active tieback and NiTi coil spring groups.

The GCF flow rate and composition vary with the severity of the inflammation during periodontal disease[24] and the same variation in the flow rate, and composition is seen during active tooth movement by orthodontic forces.[25] In our study, we noticed that the amount of GCF production increased in both active tieback and NiTi coil spring groups after application of force.

The levels of interleukin (IL)-1β, substance P,[26] TNF-α,[27] MMPs, LDH,[7] and tissue inhibitor matrix metalloproteinases[28] in the GCF reflect the biologic activity in the periodontium during orthodontic tooth movement. Heavy interrupted forces induce a rapid release of TNF but it lasts for very short duration.[5] The patients with chronic periodontitis showed an elevation in serum high-sensitivity CRP levels compared to healthy subjects.[29] Osteoprogenitor is one of the key mediators responsible for alveolar bone remodeling during tooth movement.[30] There was a peak of cytokine levels prostaglandin E2 in the GCF at 24 h during orthodontic treatment.[31] The concentration of leptin as an inflammatory mediator in GCF which is decreased by orthodontic tooth movement.[9] There was no significant elevation of CRP, TNF-α, and IL-6 at any of the time points during orthodontic treatment.[32]

It has been hypothesized that NiTi coil spring produces more biologically accepted low, constant force which is responsible for the rapid rate of tooth movement.[33] Dixon *et al*. showed the rate of space closure by active ligatures, E-chain and NiTi spring were 0.35 mm/month, 0.58 mm/month, and 0.81 mm/month respectively.[34] In our study, we found the rate of space closure by active tie back and NiTi coil spring were 0.6 mm/month and 0.8 mm/month respectively, the values of NiTi coil spring matched with above-mentioned study.

The limitation of this study is that it was a short-term study (2 weeks). A long-term study would give more conclusive results. This study compared PTX-3 level between active tie backs and NiTi coil springs, both of which are low force methods of canine retraction, a study comparing PTX-3 levels with higher force would be helpful.

CONCLUSION

The study concluded PTX-3 levels increase from 1 h after commencement of canine retraction and reached peak at 1 day, followed by decreased at 1 week and 2 weeks in both active tie back and NiTi coil spring groups. In the active tieback group, PTX-3 levels reach closer to the baseline level at the end of 2 weeks. Similar results are obtained in NiTi coil spring group, but PTX-3 levels do not approximate the baseline level. These findings suggest that PTX-3 is involved in the aseptic inflammation and periodontal remodeling in response to orthodontic forces and NiTi coil spring showed a greater rate of space closure than active tie back. This study was confined to intervals of 1 h, 1 day, 1 week, and 2 weeks, however, a study design incorporating sample collection and analysis at shorter intervals could be more comprehensive. Furthermore, multicenter trials with larger sample size would provide more insights into the PTX-3 levels.

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Nil.

Conflicts of Interest

There are no conflicts of interest.

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