

Evaluation of levels of Interleukin-1b, intensity of pain and tooth movement during canine retraction using different magnitudes of continuous orthodontic force

Rajeshwar Singh¹, Poonam K. Jayaprakash², Ankit Yadav³, Meeta Dawar³, Harpreet Grewal⁴, Amit Mishra⁵

¹Department of Orthodontics and Dentofacial Orthopedics, Mekelle University, Mekelle, Tigray, Ethiopia, ²Department of Orthodontics and Dentofacial Orthopedics, Kothiwal Dental College and Research Center, Mora Mustaqueem, Moradabad, Uttar Pradesh, ³Department of Orthodontics and Dentofacial Orthopedics, Yadav Dental and Orthodontic Centre, New Delhi, ⁴Professor and Head, Department of Dentistry, University College of Medical Sciences and Guruteg Bahadur Hospital, Dilshad Garden, Delhi, ⁵Post Graduate Student, Mithila Minority Dental College, Darbhanga, Bihar, India

ABSTRACT

Aim: The present study was conducted for the evaluation of Interleukin (IL)-1b levels in human gingival crevicular fluid (GCF), intensity of pain, and the amount of tooth movement measured during canine retraction using different magnitudes of continuous orthodontic force. **Materials and Method:** A statistically significant number of subjects were included for the study ($n = 16$, 6 male subjects and 10 female subjects). The age ranged from 18 to 24 years and all were diagnosed with Class I bimaxillary protrusion. They underwent first premolar extractions prior to participating in the study. The maxillary cuspids were then retracted using a continuous force of either 50 or 150 g. This was executed using nickel-titanium coil springs on segmented archwires. The opposite counterpart, that is, mandibular cuspid was used as control. GCF was then drawn from the distal aspect of each tooth at defined time intervals. This was followed by the assessment of IL-1b concentrations, pain intensity, using the visual analogue scale (VAS), and the amount of tooth movement. ANOVA test, Friedman test, and paired t-tests were used for comparisons of IL-1b in GCF, the plaque and gingival indices, and the efficiency of tooth movement on pain perception, respectively. **Results:** The 150 g group showed the highest level of IL-1b concentration at 24 h from baseline and at 2 with significant differences compared with the control group ($P < 0.05$). The mean VAS score of pain intensity from the 150 g force was significantly greater than from the 50 g force at 24 h ($P < 0.01$). **Conclusion:** No significant difference in the amount of tooth movement was found between these two different magnitudes of continuous force at 2 months. A 50 g force could effectively induce tooth movement similar to 150 g with less pain and less inflammation.

Keywords: Bimaxillary protrusion, gingival crevicular fluid, interleukin (IL)-1b

Introduction

Tissue remodeling is facilitated by orthodontic forces which occurs mainly as a reaction of tissues to some type of mechanical

Address for correspondence: Dr. Poonam K. Jayaprakash, Department of Orthodontics and Dentofacial Orthopedics, Kothiwal Dental College and Research Center, Mora Mustaqueem, Moradabad - 244 001, Uttar Pradesh, India.
E-mail: dr.poonamjay@gmail.com

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stimulation. A plethora of literature is available online reflecting the results of various studies conducted to determine the magnitude of optimal forces or range of force for orthodontic tooth movement.^[1-4] The appropriate forces for tooth movement of human teeth reportedly range from a force as light as 18 g to one as heavy as 1,515 g.^[2,5] This argument still exists, and no evidence-based optimal force level can be recommended in clinical

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orthodontics.^{15,61} In addition to the forces optimal for the velocity of human tooth movement, the inflammatory response and pain after orthodontic force is applied which need to be studied.

Since teeth must be moved safely as well as efficiently, it is important to determine the possible adverse effects from various magnitudes of force application, cell biology by cytokines, and patient discomfort from pain intensity. The purpose of this study, therefore, was to compare two different magnitudes of orthodontic force used for canine retraction, with regard to IL-1b secretion in GCF, efficiency of tooth movement, and pain perception. The null hypothesis tested was that there is no difference between forces of 50 and 150 g concerning these measured variables.

Subjects and Methods

Patient selection

Sixteen patients aged 18–24 years (six males, mean age 20.8 ± 1.2 years; 10 females, mean age 20.2 ± 1.6 years) participated in this study. They all met the following criteria: (1) class I molar relationship and bimaxillary protrusion with very mild crowding, especially in the posterior segment; (2) treatment plan involving extraction of all first premolars and distal retraction of the canines; (3) no evidence of periodontal or gingival disease; and (4) no history of antibiotic therapy during the previous 3 months and no anti-inflammatory drug use within 1 month before the start of the study. The reason for excluding patients with a history of recent antibiotic and inflammatory drug use was that they would affect some of the mediators released and immune functions.

Experimental design

After first premolar extractions, all subjects received oral hygiene instruction and were advised to have a soft food diet and to chew on both sides 1 month before and throughout the experimental period. To prevent plaque formation and the development of gingivitis, all subjects started rinsing with chlorhexidine mouthwash twice daily until the end of the experiment. At each appointment, the oral hygiene of each subject was evaluated using the plaque index (PI) as described by Dababneh *et al.*¹⁷ and the modified gingival index given by Lobene *et al.*¹⁸ (GI). A transpalatal arch attached on molar bands was inserted at least 1 week before the experimental procedures.

Brackets (0.022 inch slot, Ormco Corp.) and segmented archwires (0.018×0.025 inch stainless steel wire) were placed on the upper posterior teeth. The upper right and left canines of the same patient were randomly retracted using a continuous force of 50 or 150 g with nickel–titanium coil springs (Tomy®, Tokyo, Japan). The accuracy of the force was measured before canine retraction with a calibrated orthodontic force gauge (Gram Gauges, Mecmesin Asia Co. Ltd., Bangkok, Thailand). A lower right or left canine with no appliance was used as the control.¹⁹

GCF sampling

GCF was collected from the distal site of the experimental and control canines before retraction (baseline) and after retraction at 1 and 24 h, 1 week, 1 month, and 2 months without any reactivation of the coil spring. A paper strip (Periopaper; Proflow™ Incorporated, Amityville, New York, USA) was carefully inserted 1 mm into the gingival crevice on the distal side and left there for 30 s¹¹⁰ [Figure 1]. After an interval of 90 s, a second strip was carefully placed at the same site. The absorbed fluid volume was measured with a Periotron 8000 (Proflow™ Incorporated). The two periopapers of each sample site were pooled into a sealed tube and immediately frozen at -80°C .

The periopapers in each tube were eluted with 100 ml of 0.05 M TrisHCl buffer (pH 7.5) and centrifuged at 5000 g, 4°C , for 20 min. A further 50 ml of buffer was then applied, and the procedure was repeated. Subsequently, the supernatants were placed in a new tube and prepared for measurement of protein and IL-1b concentrations.

Protein assay and IL-1b determination

Protein concentrations of each sample site were measured by BCA Assay with bovine serum albumin as a standard. IL-1b levels were determined using the enzyme-linked immunosorbent assay. Total IL-1b was calculated in picograms, and IL-1b concentration in each sample site was calculated from the amount of IL-1b divided by the total protein content in GCF samples (picograms/milligrams of total protein).

Intensity of pain

For evaluation of pain intensity, all subjects were instructed to place a mark on a 100 mm VAS, corresponding to their current level of spontaneous pain intensity, including a feeling of discomfort for the right and left experimental canines separately

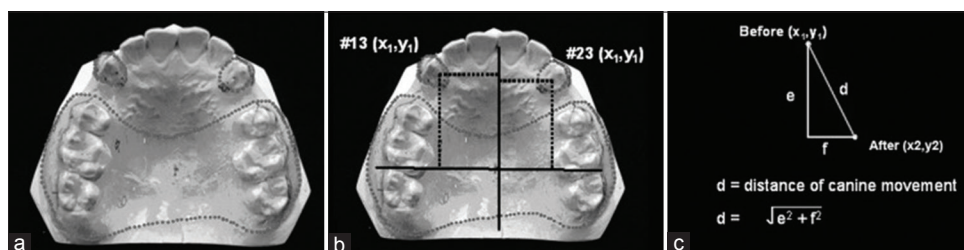


Figure 1: (a) Templates of the canines and posterior segments; (b and c) Calculation of linear changes in the position of the canines before (x_1, y_1) and after canine retraction (x_2, y_2) , d is the distance the canine moved from the start of treatment to 2 months

as well as the control tooth at all experimental time periods without any stimulation. The left end of the line was given a VAS score of 0, indicating no pain, and the right end 100, indicating maximum pain. The distance from the left side to the mark indicating pain intensity was measured three times and averaged.

Determination of the amount of tooth movement

Dental models of all subjects taken before and at 2 months were evaluated with a measuring microscope.

Statistical analyses

Data analysis was performed using the Statistical Package for Social Sciences version 14.0 (SPSS Inc., Chicago, Illinois, USA). Means and standard deviations of total protein and IL-1b concentrations from the GCF samples of all groups were calculated. For comparison of the protein or IL-1b concentrations at each observation time point within each group, repeated measures one-way analysis of variance (ANOVA) was performed. One-way ANOVA was used for comparison of concentrations of protein and IL-1b among the groups and Friedman test for comparisons of the PI and modified GI among the groups. A paired *t*-test was used for comparing VAS scores of pain intensity or the amount of canine movement between the 50 and 150 g force. The significance level was set at $P < 0.05$.

Results

All subjects showed good gingival and periodontal status at all experimental time points with no significant difference in PI and modified GI scores [Figures 2 and 3].

GCF volumes showed no significant difference among or within groups at any time point [Table 1]. The mean value of total protein concentrations in the GCF samples of all groups was approximately 12 mg/ml at all-time points (data not shown).

IL-1b concentrations in the 50 and 150 g groups increased, with the greatest mean amounts at 24 h, declined to approximately

normal levels during 1 week to 1 month, and increased again at 2 months [Table 2]. No significant difference was found between the two experimental groups ($P > 0.05$) in Canine retraction after 2 months of application of continuous orthodontic forces of 50 and 150 g [Figure 4]. Significant differences were found between the control and a force of 150 g at 24 h and 2 months ($P < 0.05$) [Table 3].

Discussion

In this study, an attempt was made to evaluate the efficacy of different amounts of orthodontic force (50 and 150 g) for tooth movement in conjunction with levels of IL-1b as well as intensity of pain because a force of 100–200 g has been recommended for canine retraction.^[11]

Table 1: Mean ± standard deviation (SD) of gingival crevicular fluid volumes for the control and experimental groups (average volume of two periopapers in microlitres; n=16)

Groups	Statistics	Before	1 h	24 h	1 week	1 month	2 months
Control	Mean	0.45	0.43	0.37	0.28	0.37	0.33
	SD	0.29	0.29	0.24	0.21	0.26	0.21
50 g	Mean	0.41	0.32	0.33	0.32	0.45	0.42
	SD	0.23	0.24	0.30	0.16	0.34	0.25
150 g	Mean	0.38	0.42	0.40	0.34	0.34	0.42
	SD	0.22	0.37	0.41	0.16	0.21	0.26

No significant difference among or within the groups ($P > 0.05$)

Table 2: Interleukin-1b concentrations (picograms/milligrams of total protein) in the gingival crevicular fluid samples of the three groups (n=16)

Groups	Statistics	Before	1 hour	24 hours	1 week	1 month	2 months
Control	Mean	0.054	0.056	0.041 ^a	0.051	0.061	0.030 ^b
	SD	0.044	0.050	0.045	0.052	0.080	0.030
50 g	Mean	0.059	0.052	0.073	0.058	0.051	0.069
	SD	0.064	0.080	0.129	0.053	0.038	0.078
150 g	Mean	0.054 ^c	0.073	0.112 ^{a,c}	0.068	0.078	0.111 ^b
	SD	0.048	0.068	0.095	0.073	0.119	0.148

Significant differences between ^athe control and 150 g group at 24 hours, ^bthe control and 150 g group at 2 months and ^cthe 150 g group before and at 24 hours ($P < 0.05$)

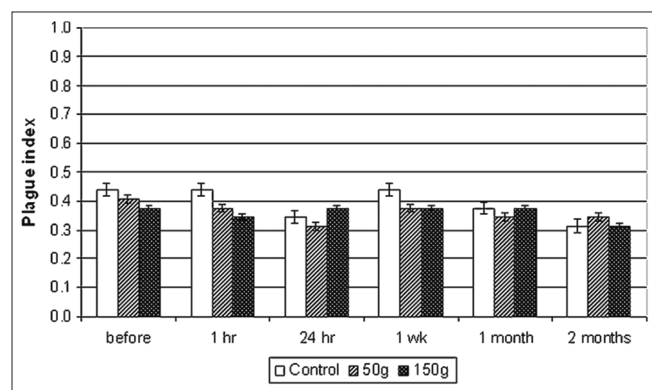


Figure 2: Plaque index score for the control experimental groups ($n = 16$). There was no significant difference among or within the groups ($P > 0.05$)

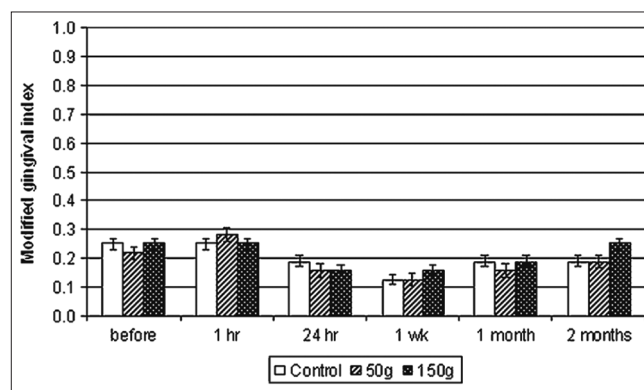


Figure 3: Modified gingival index score for the control and experimental groups ($n = 16$). There was no significant difference among or within the groups ($P > 0.05$)

Table 3: Means±standard deviation (SD) of visual analogue scale scores of pain intensity from canine retraction forces of 50 and 150 g (n=16)

Groups	Statistics	1 hour	24 hours	1 week	1 month	2 months
50 g	Mean	12.24	20.24 ^{a,b,c}	8.05 ^b	9.44 ^c	10.97
	SD	15.33	24.11	12.18	19.06	18.50
150 g	Mean	18.84 ^d	35.15 ^{a,d,e,f,g}	8.09 ^c	10.45 ^f	15.03 ^g
	SD	18.19	16.89	10.84	16.79	22.02

Significant difference ^abetween the 50 and 150 g groups ($P<0.01$), ^bwithin the 50 g group at 24 hours and at 1 week ($P<0.05$), ^cwithin the 50 g group at 24 hours and at 1 month ($P<0.05$), ^dwithin the 150 g group at 1 and 24 hours ($P<0.01$), ^ewithin the 150 g group at 24 hours and at 1 week ($P<0.01$), ^fwithin the 150 g group at 24 hours and at 1 month ($P<0.01$) and ^gwithin the 150 g group at 24 hours and at 2 months ($P<0.01$).

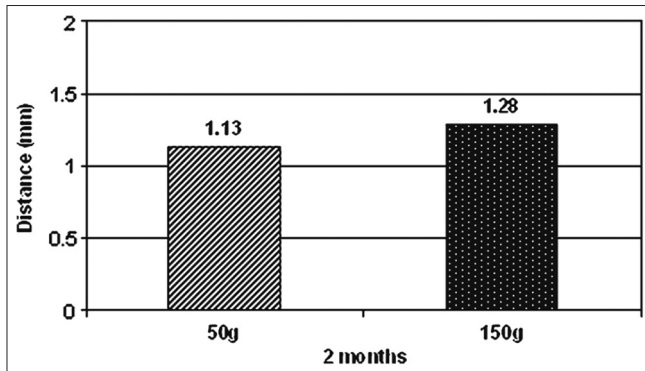


Figure 4: The mean amounts of canine retraction after 2 months of application of continuous orthodontic forces of 50 and 150 g. No significant difference was found between the two experimental groups ($P > 0.05$)

GCF collection, which is a noninvasive method that has been widely used for analysis of human tooth movement, enables easy detection of various biochemical markers^[12] (Uematsu *et al.*, 1996). Because the level of IL-1b in GCF increases with plaque accumulation and gingival inflammation, all subjects were instructed to maintain good oral hygiene practices throughout the period of the study. The PI and GI results for all subjects showed no sign of gingival inflammation or significant changes at any time point. Moreover, as there was no change in GCF volume, this demonstrated good gingival health throughout experimental period.

Interestingly, there was no significant difference between the mean amount of canine movement with forces of 50 and 150 g at 2 months, implying that force magnitudes less than 100 g could produce the same rate of tooth movement as a greater force.^[3,13] Iwasaki *et al.*^[2,14] used continuous average forces of 18 and 60 g for canine retraction and found that effective tooth movement could be produced with lower forces and that the lag phase was eliminated.

The immediate painful response from initial orthodontic force has been reported to be due to the development of an acute inflammatory process and changes in blood flow in the PDL.^[15] To evaluate pain intensity, a VAS was used as this method has been found to be valid and reliable in previous research.^[16,17] In this study, because of the well-aligned posterior teeth, canine

retraction by continuous coil springs could be performed immediately after placement of brackets and segmented arch wires. The maxillary first molar bands with the transpalatal arch had been placed more than 1 week earlier to ensure that pain from the band phase had subsided.^[18] The highest pain intensity was found in the 150 g group at 24 h, similar to other studies,^[19,20] while pain in the 50 g group was significantly less.

In the present study, at 24 h, IL-1b concentration from a force of 150 g showed the highest data, which was consistent with the reported pain. Thus, the concentration of IL-1b was to some extent related to the pain intensity. It could be considered that there might be a concentration of IL-1b, which induced sufficient tooth movement but not strong pain. A force of 50 g could be considered optimum for canine retraction.

Conclusions

A continuous force of 150 g resulted in significantly higher IL-1b levels at 24 h and after 2 months of initial canine tooth movement when compared with the control teeth. A continuous force of 50 g produced significantly less pain intensity at 24 h compared with a 150 g force. Both forces resulted in movement of the canines after 2 months, but without a statistically significant difference. A continuous force of 50 g could effectively induce canine movement similar to a 150 g force, but with less pain.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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