Research Article

Comparison of Osteoclast Count and Amount of Orthodontic Tooth Movement in Flap Corticotomy and Flapless Bur Decortication: A Preclinical Study on Rats

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Abstract

Background: Flapless bur decortication is a minimally invasive approach that can be performed as an adjunct to orthodontic treatment to expedite tooth movement.

Objective: The aim of our study was to compare amount of orthodontic tooth movement and osteoclast count in flap corticotomy and flapless bur decortication using 20 cN force in rats.

Methodology: The study was performed at animal house, Postgraduate Medical Institute, Lahore after ethical approval from institutional review board. A total of 36 male, 10-12 weeks old, (200±20 gm) Sprague-Dawley rats were randomly divided in three groups: (1) appliance only (control, n = 12), (2) flap corticotomy (FC) with appliance (n = 12), (3) flapless bur decortication (FLBD) with appliance (n = 12). Rats were euthanized at 14 days. Initial and final tooth movement were recorded using digital caliper. Osteoclast count was performed using hematoxylin-and-eosin staining method.

Results: The amount of orthodontic tooth movement and osteoclast count were significantly higher in flap corticotomy and flapless bur decortication groups than control *($P < 0.05$)*. However, no significant differences were found in mean tooth movement *($P = 0.99$)* and mean osteoclast count *($P=0.98$)* between flap corticotomy and flapless bur decortication.

Conclusion: Within the limit of current study, flap corticotomy and flapless bur decortication did not show a significant difference in the amount of orthodontic tooth movement and osteoclast which suggest that flapless bur decortication can be used to accelerate tooth movement.

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Key Words: Osteoclast, tooth movement techniques, orthodontic appliance, rats, Sprague-Dawley.

Introduction

Orthodontists are constantly seeking novel approaches to accelerate tooth movement in order to reduce treatment duration for adults. The prolonged duration of orthodontic treatment causes irritation among adults with increasing risk of dental caries, root resorption and gingival recession. In response to these factors, invasive and non-invasive adjunctive treatment approaches have been investigated to minimize treatment duration.[1] Non-invasive techniques to expedite tooth movement include mechanical vibration, low-level lasers (LLL) and low intensity pulsed ultrasound (LIPUS).[2] Additionally, surgical approaches associated with orthodontic tooth movement include invasive techniques such as flap corticotomy (FC) and less invasive techniques like bur decortication or perforations, peizocision and corticision.[3,4] While corticotomies have successfully been used to accelerate tooth movement, post-operative complications and extensive surgical procedures remain inevitable, thus patient's acceptance for invasive procedures is low.[5,6] In order to reduce trauma, time and post-operative morbidity, modified corticotomies such as piezocisions, corticisions and mini-implant micro-osteoperforations were performed without raising mucoperiosteal flap. Nevertheless, these procedures require precision, are expensive and difficult to perform by orthodontists in clinics.[7,8]

The accelerated orthodontic tooth movement is directly related to increased regional acceleratory phenomenon (RAP). The RAP in the presence of physical stimulus or bone injury increases bone turnover and decreases bone density.[9] There is evidence that adjunctive surgical interventions induce RAP and decrease bone resistance to expedite orthodontic tooth movement, however, the opinions are still controversial.[10,11]

Flapless bur decortication (FLBD) is a minimally invasive technique that does not require flap elevation and can be performed by orthodontists in clinical settings. Previous clinical reports and experiments in animals suggested that minimally invasive procedures can expedite tooth movement.[12,13] However, the use of contralateral side as control, small sample size, increased number of perforations and use of either light or heavier force (10 g, 25 g, 50 g 150 g) were the major drawbacks of these models.[14,15,16] While comparative data on accelerated tooth movement and osteoclast count using flap corticotomy and other minimally invasive procedures are available, literature comparing flap corticotomy and flapless bur decortication is lacking.

Therefore, the aim of our study was to compare osteoclast count and amount of orthodontic tooth movement in flap corticotomy and flapless bur décortication at 14 days using 20 cN orthodontic force.

Methods

The experiment was approved by ethical committee of Postgraduate Medical Institute, Lahore and performed in accordance with institutional and ethical regulation for animal experiment.

Thirty-six healthy male, 10-12 weeks old, (200±20 gm) Sprague- Dawley rats were included in the experiment. Rats were allocated in three groups by simple random sampling. Twelve rats were placed in each group as follows: (1) appliance only (control, n=12), (2) flap corticotomy (FC) with appliance (n=12), (3) flapless bur decortication (FLBD) with appliance (n=12). The rats were kept in cages at a temperature of 24± 0.5 °C and were fed on rat food and water ad libitum. The left side of maxilla was used as experimental side in all three groups. The right maxillae did not receive any treatment in order to keep the nourishment capacity of rats unhampered. After the procedure, all rats were euthanized at 14 days. Power calculation was based on previously published study.[17] A total of 36 rats were determined with 12 rats in each group in order to obtain reliable estimates of osteoclast count and orthodontic tooth movement.

All procedures were performed under general anesthesia by administering 8 mg/kg ketamine and 5 mg/kg xylazine in the intraperitoneal region. Rats were weighed at day 0 and day 14. The application of orthodontic force and corticotomy procedures were performed at day 0 and osteoclast count and orthodontic tooth movement were observed at day 14. Initially, at day 0, the initial distance (T1) between mesial occlusal pits of upper left molars (first molar and second molar) was recorded using digital Vernier caliper by one operator in all three groups.

Flap corticotomy was performed in group 2 as previously described.[17] Briefly, flap was raised on the palate adjacent to the left first molar and extended on mesial side to allow access for perforations. The cortical bone was perforated 5-

mm mesial to the left maxillary first molar to a depth of 0.25 millimeter and 0.25 millimeter in diameter using one-millimeter tungsten carbide round bur attached to an automated high-speed drill (12,000 rpm) under water irrigation. Three cortical perforations were placed each with a distance of 1-3 mm. Primary closure was achieved using silk suture. The sutures were removed 3-4 days after the procedure. In group 3, flapless bur decortication was performed. The cortical bone was perforated through soft tissue without raising flap under copious water irrigation using tungsten carbide round bur attached to high speed hand piece. In group 3, similar to group 2, three cortical perforations, each with a distance of 1-3 mm were placed, 5-mm mesial to the left maxillary first molar to a depth of 0.25 millimeter and 0.25 millimeter in diameter. After the procedure, cotton pellets were placed to control bleeding. In control group, neither flap corticotomy nor flapless bur decortication was performed.

An orthodontic appliance was secured in all three groups as described previously.[18] Briefly closed coil spring was fixed through lace back wires (0.9 mm) in interdental spaces between first and second molars and between two incisors. Orthodontic closed coil spring of nickel titanium (NiTi) with diameter of 0.010×0.030, 5 mm long and constant force of 20 cN was used as an orthodontic appliance. The appliance was placed for 14 days to produce an orthodontic tooth movement in a molar tooth. The height of the appliance was maintained below the occlusal plane. Rats were monitored on daily basis for change in weight, pain, and discomfort and appliance dislodgment. On day 14, all rats were euthanatized by inhalation of carbon dioxide followed by cervical dislocation. Left maxillae were dissected and the final distance (T2) was measured from mesial occlusal pits of upper left first to second molar. The amount of tooth movement was calculated by subtracting initial distance (T1) from final distance (T2). The measurements were repeated twice by one operator and the mean value in millimeters was recorded.

The hemisected maxillae were fixed in 10% fixative solution (formalin), demineralized in formic acid (50%) and sodium formate for 6-8 weeks, dehydrated in alcohol series and embedded in paraffin. Following embedding in paraffin, 5-micrometer thick sections were cut using microtome and slides were prepared using hematoxylin and eosin (H & E) staining. To locate and count osteoclast, the mesial-surface (compression side) of a mesial-root of maxillary left first molar with appliance position was identified. The histomorphologic study and counting of osteoclasts were performed manually under light microscope (Nikon Eclipse E600, 400x magnification) by histopathologist who was blinded to study. The counting of osteoclasts was repeated twice by same histopathologist and mean value was registered (Figure 1).

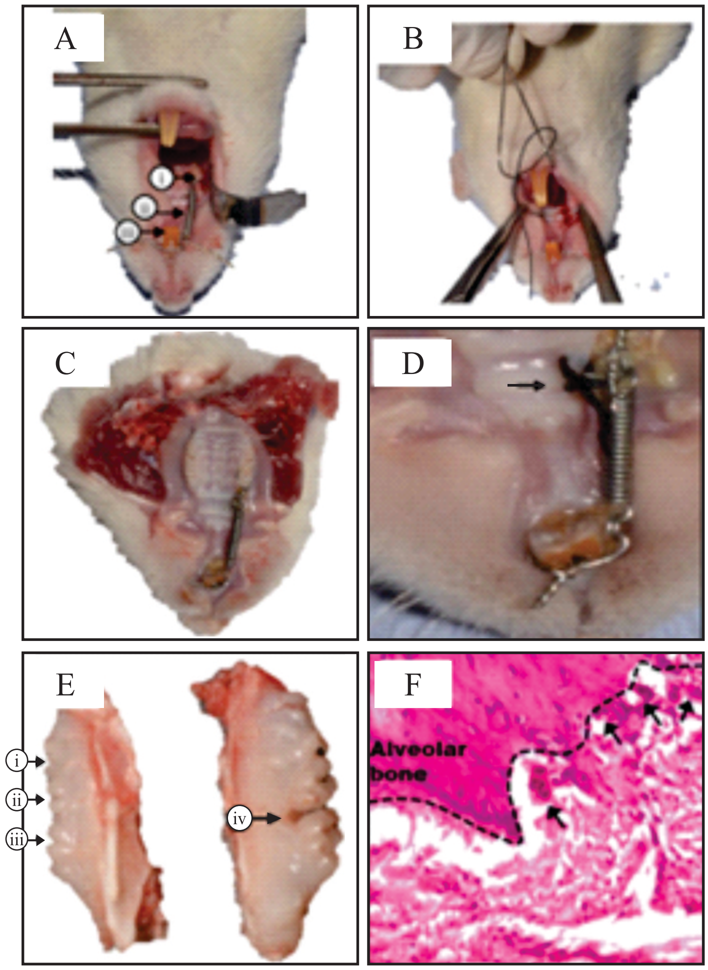

Figure 1: *Flap Corticotomy and Flapless Aur Decortication*

(A) Photograph to demonstrate fixation of closed coil spring through lace back wire, i: maxillary 1st molar; ii: closed coil spring; iii: incisors.

(B) Flap suturing post-flap corticotomy.
(C) Dissected maxilla of experimental group with flapless bur decortication with appliance.
(D) Maxilla of experimental group with flap corticotomy (suture indicated)
(E) i; maxillary 3rd molar; ii: maxillary 2nd molar (M2); iii: maxillary 1st molar (M1); iv: distance between M1 and M2
(F) Microphotograph x400 showing H & E staining of tissues obtained from rats undergoing flapless bur decortication. Arrows indicate osteoclasts, dotted margin indicates boneresorption-margin.

Statistical Analysis

Data were entered and analyzed using SPSS version 24. Shapiro-Wilk test was used to check normal distribution of data. The orthodontic tooth movement and osteoclast count were normally distributed. One-way ANOVA was used to compare the means between three groups. Post-hoc Tukey test was used to analyze which groups were different. P value less than 0.05 was considered to be statistically significant ($P < 0.05$).

Results

All rats during experiment remained healthy and had a slight increase in body weight. None of the rats lost appliance during experiment in all three groups.

At day 14, a significant increase in amount of tooth movement ($P < 0.05$) was observed in experimental groups that received flap corticotomy (FC) with appliance (M = 0.51±0.11 mm) and flapless bur decortication (FLBD) with appliance (M = 0.52±0.16 mm) compared to the control group that received appliance only (M = 0.22±0.05 mm). However, no significant difference was observed in the amount of tooth movement between FC and FLBD groups (Table 1 and Table 2).

The osteoclast count was significantly higher in both FC and FLBD groups than in control group ($P < 0.05$) at day 14. However, no significant difference was found between FC and FLBD groups ($P = 0.98$) (Table 1 and Table 2).

Table 1: *Mean Tooth Movement and Osteoclast Count in Three Groups at 14 Days*

	Groups	N	Mean	SD	95% Confidence Interval for Mean		Minimum	Maximum	*P*-value
					Lower Bound	Upper Bound			
	Control	12	0.225	0.052	0.1919	0.2581	0.12	0.29	0.001*
Mean tooth movement (mm)	FC	12	0.515	0.119	0.4400	0.5917	0.30	0.71	0.001*
	FLBD	12	0.522	0.159	0.4214	0.6236	0.26	0.83	0.001*
	Total	36	0.421	0.181	0.3596	0.4826	0.12	0.83	
	Control	12	14.006	0.842	13.4717	14.5417	12.40	15.00	0.001*
Mean OC count	FC	12	25.714	1.931	24.4875	26.9408	22.30	28.70	0.001*
	FLBD	12	25.821	1.798	24.6788	26.9645	22.30	28.70	0.001*
	Total	36	21.847	5.833	19.8737	23.8213	12.40	28.70	

*P-value < 0.05
Abbreviations: FC = flap corticotomy, FLBD = flapless bur decortication, mm = millimeter, OC= osteoclast

Table 2: *Multiple Comparison Test of Mean Orthodontic Tooth Movement and Osteoclast Count Between Groups*

Variables	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
	Control	FC	-.29083*	0.04847	0.001*	-0.4098	-0.1719
		FLBD	-.29750*	0.04847	0.001*	-0.4164	-0.1786
Mean tooth movement (mm)	FC	Control	.29083*	0.04847	0.001*	0.1719	0.4098
		FLBD	-0.00667	0.04847	0.990	-0.1256	0.1123
	FLBD	Control	.29750*	0.04847	0.001*	0.1786	0.4164
		FC	0.00667	0.04847	0.990	-0.1123	0.1256
	Control	FC	-11.70750*	0.65286	0.001*	-3.3095	-0.1055
		FLBD	-11.81500*	0.65286	0.001*	-3.4170	-0.2130
Mean OC count	FC	Control	11.70750*	0.65286	0.001*	10.1055	13.3095
		FLBD	-0.10750	0.65286	0.985	-1.7095	1.4945
	FLBD	Control	11.81500*	0.65286	0.001*	10.2130	13.4170
		FC	0.10750	0.65286	0.985	-1.4945	1.7095

*P- value < 0.05

Abbreviations: Std error = standard error, Sig. = significance, FC = flap corticotomy, FLBD = flapless bur decortication, OC = osteoclast, mm = millimeter

Discussion:

In the current study, significant differences in the amount of orthodontic tooth movement and osteoclasts count were observed in flap corticotomy and flapless bur decortication groups than control group. These findings are in accordance with Tsai et al. who found significant orthodontic tooth movement and osteoclast count at 14 days in surgical groups than control.[14] Also, in parallel with current findings, Kim et al. found increased amount of orthodontic tooth movement in flap corticotomy and flapless micro-osteoperforation than control group.[7] Chen et al. also reported significant difference in osteoclast count and tooth movement at 14 days in corticotomy groups than control.[19] Our results are in contrast with those of Librizzi et al. who found no significant difference in tooth movement and osteoclast count in flap and flapless corticision than control group.[20] One of the plausible explanations for different findings might be related to age of rats. In the current study, adult rats (10-12 weeks old) were used contrary to young rats (6 weeks old). Age related effects in orthodontic tooth movement were previously studied by Ren et al. who found significant difference in tooth movement in both young and adult rats.[21] Moreover, Li et al. reported increased osteoclast count in both young and adult rats after application of force for 7 days, however, the osteoclast count was reduced in young rats in retention period of 7 days compared to adult rats.[22] Taken together, the bone-remodeling decreases with age which might explain why adult orthodontics is more laborious.

The present study showed no significant difference in orthodontic tooth movement and osteoclast count between flap corticotomy and flapless bur decorticateion groups at day 14. Our findings are in agreement with Kim et al. who found no significant increase in orthodontic tooth movement in flap corticotomies and flapless micro-osteoperforation at 14 days.[7] Peron et al. also reported no difference in the amount of tooth movement in corticotomy and corticision groups.[23] Librizzi et al. also showed no significant difference in amount of tooth movement and osteoclast count in corticotomy and corticision (with or without flap) groups at 21 days.[20] These comparable findings suggest that biological-bone responses might have occurred in first 14 days regardless of flap or flapless procedure. Recently, Owen et al. reported that flap alone without bone injury expedites tooth movement by reducing the amount and density of medullary bone; nevertheless, these effects were limited.[24] Meanwhile, our study results showed no significant difference in flap and flapless bur decortication that suggest similar effect for both treatment approaches.

The current results also contrast with Agrawal et al. who reported significant difference in amount of tooth movement between flap and flapless corticotomy groups at 14 days and 28 days of follow-up in humans.[25] The plausible reasons for different findings might be related to number of perforations, distance between perforation and tooth to be moved and availability of area for decortication. In the current study, three perforations, 5-mm mesial to maxillary left first molar were placed in both flap corticotomy and flapless bur decortication groups compared to the large and small area of perforations respectively in flap and flapless corticotomy sites.

It is also important to note that in the current study, rats did not lose weight during experiment suggesting the unhampered physiological activity. One of the limitations of our study was that reliability of measurements and counts was not assessed. The availability of histological sections was the strength of the study which is not feasible in other animals or humans. More studies on animals are needed to investigate different magnitude of orthodontic force, duration of experiment, number of perforations, root resorption, gingival recession in flap corticotomy and flapless bur decortication.

Conclusion:

Within the limit of current study, flapless bur decortication is comparable to flap corticotomy in terms of tooth movement and osteoclast count suggesting the use of flapless bur decortication (minimally invasive technique) to accelerate tooth movement. However, further animal researches are needed with regard to duration, magnitude of force, number of perforations, root resorption in flap corticotomy and flapless bur-decortication.

Ethical Approval: Given
Conflict of Interest: The authors declare no conflict of interest
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