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ORIGINAL ARTICLE

Histomorphometric Assessment of Vibrational Forces on the Extended Midpalatal Suture

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ABSTRACT

Objective: This study determined the effects of vibrational forces on bone formation in the retention period of an expanded mid-palatal, histomorphometric suture in a rat model. **Methods**: Twenty-four 7–8- to 8-week-old male Wistar rats weighing 190.25 ± 15.35 g were separated into groups: only expansion (G1), expansion and 30 Hz vibration application (G2), and expansion and 111 Hz vibration application (G3). After 5 days of expansion, there were 12 days of retention. All animals were sacrificed, and their premaxilla were dissected. A histological examination evaluated the number of osteoblasts, osteoclasts, and capillaries, the amount of mineralized area, and the fibrosis area. **Results**: Statistical analyses showed significant differences among the groups for all parameters. The number of osteoblasts, new bone area, fibrosis area, blood vessels, and measurements demonstrated statistically significant differences. For all histomorphometric parameters except osteoclast numbers, G3 showed more positive results than G1 and G2 regarding new bone formation ($p < 0.05$). **Conclusion**: The application of vibrational forces might stimulate bone formation in an orthopedically expanded midpalatal suture during the retention period. These applications may help prevent relapse after the expansion procedure.

Keywords: histomorphometry, rats, suture, vibrational force

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INTRODUCTION

Abnormality in tooth alignment or the dental arch relationship in sagittal, vertical, and transversal directions is called *malocclusion*. Maxillary arch deficiency, the most common transversal problem, may lead to aesthetic, functional, and respiratory problems.[1,2] The treatment method that will provide orthodontic or orthopedic improvement in enlarging the upper jaw is chosen by considering many factors such as etiology, age, the severity of the narrowness, patient compliance, and inclination of the posterior teeth.[2,3] Rapid maxillary expansion (RME) is a procedure widely used for growing children in orthodontic practices to correct posterior crossbites and maxillary narrowness.[2-4] The process comprises 2 stages. In the first active part of the process, a high amount of force is transmitted to the mid-palatal suture by an intraoral fixed appliance, which rapidly widens the apical base level of the maxilla. In the second stage, the passive part of the process, remodeling of the midpalatal suture and new bone formation, is achieved.[4,5] Although the RME method has many advantages in orthodontic treatment, complications may occur after this procedure, including external root resorption, mid-palatal suture microfractures, microtrauma of the joint, and relapse.[5-8] Of these potential complications relapse most affects the treatment process. Accelerating bone regeneration and new bone formation after maxillary suture expansion is desirable to prevent recurrence and shorten retention time.[9] Consequently, there are many studies in which different applications are made to affect bone metabolism and accelerate osteoblastic activity in the enlarged suture region during the transition to retention. These include laser tissue stimulation, ozone application, transforming growth factor, bisphosphonates, antioxidants, lactoferrin, vitamins, and the application of agents such as dietary boron.[4-17]

Vibratory forces (VF) are a non-pharmacological and non-invasive approach used to stimulate bone formation.[18-21] VF has been shown to effectively improve osteoporosis, fracture healing of bone, and osteointegration of dental implants. Studies show increased rates of bone formation and mineral apposition when cyclic VF is applied to long bones.[22-26] Moreover, vibrational force has been shown to affect new bone formation of the craniofacial sutures.[27-30] Many studies have used VF to accelerate tooth movement, but no published study currently describes the effects of different vibration frequencies during maxillary expansion.

Our aim in this experimental study was to evaluate the effects of different vibrational force values on the new bone formation histomorphometrically in the expanded area after the maxillary expansion procedure in a rat model. Considering the stimulatory effects of vibrational force on bone formation, we expect to gather considerable information about preventing or shortening the retention period after the RME procedure.

METHODS

Animals and groups

Twenty-four 7-8 weeks old male Wistar rats weighing 190.25 ± 15.35 g were used in this study. The animal experimental protocol was approved by Istanbul Mehmet Akif Ersoy Animal Experiments Local Ethics Committee (2019/29) with principles followed by the Basel Declaration, 2010. The study was carried out at the Istanbul Mehmet Akif Ersoy Experimental Research Development and Training Center. All experimental animals were kept in the same type of polycarbonate cages with 12 12-hour light and dark cycles. A standard pellet diet (Expanded pellets; Stepfield, Witham, Essex, UK) and an environment with standard temperature (23 °C) and pressure (1-atmosphere pressure) protocol were applied for all animals. The sample size was calculated at a 95% confidence level using the G Power-3.1.9.2 program.[31] The minimum sample size was found as 8 per group (24 for total sample size), based on a theoretical power of 0.80, α value of 0.05, and a standardized effect size of 1.63.15. The health status and weight changes of the animals were checked regularly throughout the study period. The initial weights of the animals were measured, and animals with body weights less than 20 percent of their initial weight were excluded from the study.

Study design - Expansion and retention protocol

A helical spring made of a 0.014-inch stainless steel wire was used for sutural expansion. Springs were activated with pliers on a grid. Intraperitoneal administration of xylazine (3mg/kg; Rompun®, Germany) and ketamine (90 mg/kg; Ketasol®, Senden-Bösensell, Germany) were used for anesthetizing the experimental animals. A hole was drilled by washing saline solution in both incisors at the gingival level, and springs were inserted into the holes buccally. Flowable composite resin (Filtek™, 3M ESPE, MN, USA) was used to fix them. The force was measured as 50g, and the springs were not reactivated until the end of the experiment. After the expansion period, the expansion spring was deactivated and fixed by flowable composite, and the retention period was started for 12 days. The gap between the maxillary incisors was measured 3 times with a digital caliper (MSI-Viking, SC, USA). T0: before expansion, T1: after expansion (5th day) and T3: after retention (17th day). The expansion spring remained passive on the teeth during the retention period. Expansion and retention periods were the same for all the animals. The animals were observed.

Application of VF treatments

At the end of the expansion period, all the animals were divided into 3 groups. Only expansion and retention protocols were applied for the control group, G1. No other application was made during the retention period. Acceledent was used to deliver 30 Hz VF for G2, and Tooth-Masseuse was used to deliver 111 Hz VF for G3. Mechanical vibration force was applied every day for 20 minutes a day during the 12-day retention period after expansion.

Histological analysis

After a 12-day period of retention, the animals were sacrificed at the same time with an overdose of Ketamine and Xylazine application. The premaxilla of the experimental animals was dissected. The dissected maxillae containing the palatal sutures were fixed in 10% neutral buffered formol for 48 hours. After fixation, the Osteosoft (Merck, Darmstadt, Germany) solution was used for decalcification. The sections are covered with Canadian balm. Randomly selected, three sections were examined. For immunohistochemical analysis, an antigen-retrieval procedure was performed. Nuclear staining (methyl green) was performed for 10 seconds to counterstain sections that were washed with distilled water. TRAP staining was performed to determine the osteoclasts. Sections were inserted into TRAP (Mountain View, CA, U.S.A.) staining solution. Sections were washed with distilled water, counterstained with nuclear staining (methyl green) for 10 seconds, and covered with Canadian balm. [32,33]

Statistical analysis

SPSS software package program for Windows (Version 25.0, IBM Corporation, Armonk, NY) was used. When the p-value was <0.05, the statistical test was determined as significant. Descriptive statistics are given as mean, minimum, maximum, and standard deviation. As the first step, the Shapiro-Wilk test was done to check the assumption of normality. Anova and Kruskal–Wallis tests were used to evaluate the differences in the number of osteoblasts, osteoclasts, and capillaries among the three groups.

RESULTS

In the control and experimental groups, all rats survived until the study's end. Mucosal infection, dehiscence, or not opening the suture were not observed in any of the animals. However, one animal in G2 and one in G3 exhibited incisor tooth fractures; therefore, those animals were excluded from the experiment. These animals were replaced with two new rats. The spring successfully achieved suture expansion, and the rats gained weight. At the beginning of the experiment, there was no median diastema (T0) in any of the rats. Therefore, 0 mm was accepted as the initial distance between the upper incisors. Distances between the incisor teeth of each group after expansion (T1) and after retention (T2) are presented in Table 1. The inter-incisor distances were similar between the experiment and the control groups and were non-significant ($p > 0.05$). Midpalatal suture width measurements showed that the suture was the expanded application of an activated spring.

A number of osteoblasts, new bone area, fibrosis area, and blood vessel measurements showed significant differences between groups in Table 2. For all investigated histomorphometric parameters, except osteoclast numbers, G3 showed better results than other groups ($p < 0.05$). In all interventions, the 111 Hz vibration application showed significantly better results than G1 and G2 in osteoblast numbers ($p = 0.00$) (Table 2 and Figure 1). The lowest number of osteoblastic cells was found in G1 (Table 2 and Figure 2). The lowest number of osteoclasts was found in the G1. There was no significant difference in the mean number of osteoclasts when compared between the groups ($p > 0.05$) (Table 2 and Figure 3).

DISCUSSION

This study was designed to demonstrate whether there is an active effect of VF on bone formation in the expansion of the midpalatal suture in rats. Relapse after the expansion procedure is a problem that orthodontists have to solve. Mode of expansion, age of the patient, gender of the patient, bone metabolism, force magnitude, retention time, ossification of the suture, and rigidity of the appliance could affect the possible relapse of RME procedure.[5-12]Although there are still many unknowns about the mechanism of recurrence, it will be beneficial in terms of accelerating bone formation in the mid-palatal suture after expansion, shortening the retention time and preserving the resulting width. There are many different approaches to accelerate new bone formation to prevent relapse after RME.[9-14]

In this study, a rat model was used to investigate the expansion of the maxilla. It was decided to conduct the study on an animal model for ethical reasons. Although the maxillary suture of monkeys and cats is closer to that of humans, the rat model was chosen because it gives clear information of bone and suture changes under stress. According to the literature, bone formation reduces with age, and intended orthopedic effects in the suture can be obtained before and during pubertal growth. 7-8 weeks-old rats were preferred in this study because obtaining palatal expansion becomes difficult after the pubertal growth period.[6-17] The sample size may differ from the sample size of some other studies. For ethical reasons, the number of the animals should be minimal. For this reason, a power analysis was performed to achieve valid scientific results in the study by using a minimum number of animals.[34]

A stainless-steel wire helical loop spring expanded the premaxillary suture in rats under general anesthesia.[5,9,11,17] Approximately a force of 50–70 g in rats was found to be enough to achieve skeletal premaxillary suture expansion, and in the current study, 50g force was used to expand all the mid-palatal sutures of the rats. According to the literature, light forces up to 20 g could not provide an opening of the mid-palatal suture; they only produced orthodontic tooth movement. In the studies carried out, researchers showed that continuous heavier forces might cause periodontal ligament necrosis and hyalinized areas of the alveolar bone, and this prevented tooth movement and opening of the suture. For this reason, 50 g force was preferred for expansion, as was done in previous studies.[8,9,11,13]

Table 1. The maxillary interincisor distance (mm) for each group after expansion (T1) and after retention (T2) (n = 8)

Group	Mean	SD	Minimum	Maximum	p*
T1					
G1-Control	3.02	0.04	2.94	3.07	0.120
G2-V30	3.06	0.09	2.98	3.25	
G3-V111	3.01	0.09	2.87	3.11	
T2					
G1-Control	3.00	0.04	2.92	3.06	0.200
G2-V30	3.03	0.06	2.99	3.16	
G3-V111	2.98	0.08	2.85	3.08	
T2-T1					
G1-Control	0.02	0.00	0.01	0.03	0.070
G2-V30	0.03	0.03	0.00	0.09	
G3-V111	0.03	0.01	0.01	0.06	

*Kruskal Wallis test, SD: Standard Deviation

Table 2. Comparison of means of variables by groups (n = 8)

	Mean	SD	Minimum	Maximum	p*
Number of osteoblasts					
G1-Control	10.66	2.16	8.00	14.00	0.000
G2-V30	11.50	3.93	7.00	17.00	
G3-V111	20.50	3.56	17.00	26.00	
Number of osteoclasts					
G1-Control	1.33	1.03	0.00	3.00	0.390
G2-V30	1.50	1.04	0.00	3.00	
G3-V111	2.16	1.16	1.00	4.00	
Number of capillaries					
G1-Control	3.50	1.76	2.00	6.00	0.000
G2-V30	5.50	1.51	3.00	7.00	
G3-V111	9.83	2.78	6.00	13.00	
Mineralized area					
G1-Control	23.77	3.37	20.55	28.16	0.000
G2-V30	30.13	5.56	20.77	35.48	
G3-V111	41.06	5.58	33.14	47.13	
Fibrosis area					
G1-Control	33.20	5.17	24.15	39.12	0.000
G2-V30	31.30	1.60	28.81	33.37	
G3-V111	21.23	3.86	15.67	27.34	
H score					
G1-Control	87.50	37.91	50.00	150.00	0.000
G2-V30	112.50	54.19	50.00	200.00	
G3-V111	229.16	45.87	175.00	300.00	

*Kruskal Wallis test, SD: Standard Deviation, $p < 0.05$

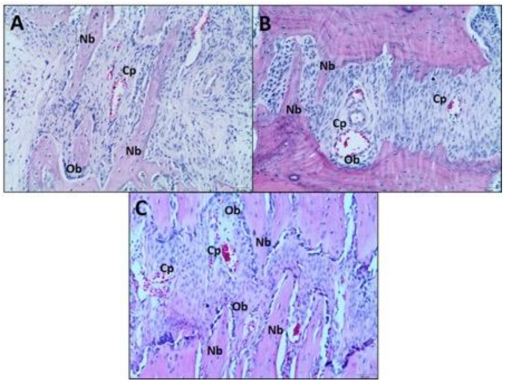

Figure 1. Hematoxylin and eosin staining photomicrographs from groups. Increased osteoblast, new bone, and capillary formation were observed in V111 Hz application G3. A: Control G1, B: V30 G2 and C: V111 G3. Nb: new bone, Cp: capillary, Ob: osteoblast.

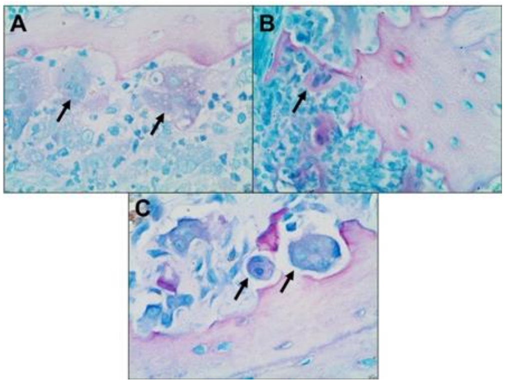

Figure 2. Representative images of TRAP-positive osteoclasts in the groups (osteoclasts indicated with a black arrow). In all figures, the magnification is indicated by scale bars. A: Control G1, B: V30 G2 and C: V111. G3.

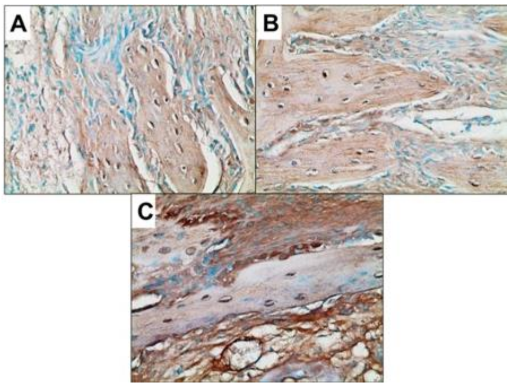

Figure 3. Representative micrographs of OCN immunohistochemical staining in control G1 (A), V30 G2 (B), V111 G3 (C) groups. In all figures, the magnification is indicated by scale bars. Methyl green is used for counterstaining.

According to the report of Burstone and Shafer, normal pre-maxillary suture in young rats measured 20 to 60 μm in thickness. 5-days opening of the suture may provide an average width of 377 ± 104 μm.[35] The mid-palatal suture was opened by SS springs that were applied buccally in this study, and measurements were done before and after the expansion procedure to show the separation after 5 days of expansion. There are studies evaluating the expansion with both measurement and histological analysis.[5,11,12] In our study, the distance between the maxillary incisors was measured with a caliper at the start and end of the expansion.

Researchers have done many studies to increase bone formation in palatal expansion and prevent relapse by the use of different pharmacological and biochemical agents in rats.[4-17] Short mechanical loading provides a response on bone tissue.[36] Due to this feature of bone tissue, researchers have attempted to accelerate bone remodeling through vibrational forces. This study chose vibrational force due to its noninvasive and easy application.

VF has been shown to have an effect of improving osteoporosis, fracture healing, and implant osteointegration.[22-26] Studies have been done to evaluate the effect of VF on the acceleration of tooth movement, but there have been no studies aimed at investigating the effects of different values of VF for maxillary expansion. The objective of this study is to investigate the effects of VF on maxillary expansion. It is recommended to allow bone formation and maturation for a period of six months or longer to minimize relapse. Vibrational forces have been used as a noninvasive approach to stimulate and accelerate bone formation. Cyclical VF delivered to long bone results in increased mineral apposition and bone formation. Cyclic Vibrational Forces have been shown to increase bone volume density of the alveolar bone and affect new bone formation of the craniofacial sutures.[18,19,27] However, no study has been done to investigate and evaluate bone formation during RME.

The frequency of VF ranged from 1Hz to 200Hz with a wide range of load magnitude and loading regimens. A variety of VF parameters and loading regimens need to be studied more because no literature is available on this subject. The thesis study chose the VF frequency of 60 Hz delivered three times a week for this experiment.[21] In our study, 30 Hz and 111 Hz were chosen as low and high frequencies, respectively, and VF treatment applications were made on a daily basis.

Two examiners conducted the histologic evaluation, which was blinded to the identity of the sections. The numbers were averaged. In this study, we evaluated the number of osteoblasts as statistically higher (1.92-fold) in the 111 Hz vibration group than in the control group. Our findings correlated with the study results, which evaluated vibration in the RME procedure. Vibrational forces may affect the retention process by causing changes in the number and structure of bone cells in the midpalatal suture. Based on the present study, good levels of interaction and bone formation were obtained in the application of 111 Hz vibration.[21] This study is the report of the positive effects of vibrational force on RME, and it is also the first study to examine the effect of two different vibration values. At the time of completion of this study, no published study was found in the literature other than a thesis on the effect of using VF to promote new bone formation in the RME. However, conducting experimental studies using different vibration values before clinical studies are carried out would be beneficial.

CONCLUSION

The application of vibrational force can stimulate bone formation in the expanded premaxillary suture during the expansion and the early phase of the retention periods. This study is the first to present data indicating that different values of vibrational forces can improve new bone generation in the expanded mid-palatal suture in rats. As this is an experimental animal study, more clinical studies should be conducted to recognize which vibration level and application time are more effective.

CONFLICT OF INTEREST

All authors declared no conflict of interest.

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