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Effect of local injection of 1,25-dihydroxycholecalciferol on the velocity of Orthodontic Tooth Movement and Bone Density

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ABSTRACT

Aims: The present study designed to evaluate the effect of of 1,25-Dihydroxycholecalciferol on the velocity of tooth movement and bone density secondary to application of orthodontic forces.

Materials and methods: This study used 15 male albino rabbits were randomly divided into three groups of five rabbits each according to time of sacrifice (1,2 and 3 weeks) . In each side, an orthodontic open coil spring with two bands placed on the lower central incisors to deliver 50 g force for orthodontic tooth movement. Using a split-mouth study design, the mandible of each animal was randomly assigned as a 1,25 DHCC side in which 20ul intraligamentous injections of a solution of 1,25Dihydroxy cholecalciferol in dimethylsulfoxide into the distal side of the right incisor while the left central side received 20ul of dimethylsulfoxide only . The injection was repeated three times every week for a period of three weeks. Amount of tooth movement and bone density were measured from digital radiograph using planmeca Dimaxis Pro X-ray machine with dimaxis classic imaging software at ten time points .

Results: The 1,25 DHCC side had increased OTM when compared to its contra-lateral control side at all time intervals . Although the 1,25 DHCC side had higher bone density than the control side in all sites and at all time intervals but the difference was insignificant. the bone density increased with time ,more over the bone density increased progressively from cervical to apical area in both sides ,in addition the medial bone density was higher than lateral bone density.

Conclusions: Local administration of 1,25 DHCC is clinical and cost effective in accelerating orthodontic tooth movement.

Key words: bone density, 1,25- dihydroxycholecalciferol , velocity of orthodontic tooth movement.

Introduction

Orthodontic treatment especially from a patient's point of view has two major drawbacks of being a lengthy and costly procedure, several studies have suggested that there might be ways to increase cellular activities with agents more potent than mechanical force alone.(1-3) 1,25 dihydroxycholecalciferol (1,25DHCC) is the most active hormonal form of 1,25 DHCC. It regulates calcium and phosphate serum levels by promoting their intestinal absorption and reabsorption in the kidneys. Furthermore, it promotes bone deposition and inhibits PTH release.(4) The effect of 1,25DHCC on orthodontic tooth movement(OTM) has been studied by several authors Collins and Sinclair (5) as well as Kale et al. (6) have reported that the local administration of 1,25 DHCC increases the rate of tooth movement(about 60%) in cats and rats respectively; they have emphasized that administration of 1,25 DHCC results in a good balance between deposition and resorption of bone and well- modulated bone turnover compared to prostaglandin administration ·Moreover, in vivo studies appearing in the orthodontic literature have shown increased levels of orthodontic tooth movement upon daily periodontal ligament(PDL) injections of 1, 25-DHCC.(6) In a rat model, repeated injections of 1, 25-DHCC with concurrent application of tooth movement decreased the mineral appositional rate (MAR)on the compression side and increased it on the tension side. Al-Hasani et al.(2011)(7) have observed that the dose of 25 picograms (pg) 1,25- dihydroxycholecalciferol produced about 51% faster rate of experimental canine movement compared to control, while each of the 15 pg and 40 pg doses resulted in about 10% accelerated OTM.

Orthodontic treatment not only moves teeth to better positions but also induces a response in the alveolar bone. Changes in density of the alveolar bone around teeth during orthodontic treatment are difficult to observe and measure. Some authors [(8-14)] have indicated that the alveolar processes in both jaws became denser after treatment, while others found reduction of alveolar bone density by orthodontic treatment[(15,16)]. The disagreement on the effect of orthodontic tooth movement on alveolar bone density is likely caused by the difference in the timing that the observations were made since orthodontic treatment began. To date, however there are few studies on the effect of 1,25DHCC on the orthodontic tooth movement but there is no study available about the effect of 1,25DHCC on bone density using digital radiograph . Thus the aim of this study was to investigate the effect of 1,25DHCC on 1)the amount and velocity of orthodontic movement of the mandibular incisors and 2) the bone density at different time intervals and different sites.

Materials and Methods

▪ Animals:

This study included fifteen male albino rabbits were randomly divided into three groups of five rabbits each according to time of sacrifice (1,2 and 3 weeks). All animals were housed in a 12-h light/dark environment at the same conditions of good ventilation, adequate stable diet temperature and humidity .They had free access to water and food throughout the experiment . Their average weight 1400 grams ranged between1250 grams and 1500grams at the beginning of the experiment. The animals were randomly divided into three equal sides according to the time of sacarifce (1,2 and 3 weeks) .

▪ Study design:

Using a split-mouth study design, the mandible of each animal was assigned as experimental (right central) side and control (left central) side . In the experimental side, 20ul intra ligamentous injection of a solution of 1,25-dihydroxycholecalciferol in dimethylsulfoxide [DMSO, Gainland Chemeical Company(GCC), United Kingdom) was given by intraligamentary (periodontal) injection locally into the distal side of the right mandibular incisor using a disposable tiny gauge needle and syringe. The left side received 20ul of dimethylsulfoxide only which was used as a vehicle as described in previous studies.[3,11,12] The injection was repeated three times every week for a period of three weeks (total of 9 times within the 22-day experimental period). [11]

▪ Orthodontic appliance:

After anaesthetization with intramuscular injection of a mixture of xylazine (10.0mg/kg of body weight) and ketamine (40 mg/kg of body weight),a fixed orthodontic appliance constructed from two stainless steel adapted orthodontic bands on the right and left incisors were cemented to the mandibular incisors with zinc oxyphosphate cement. Orthodontic nickel titanium open coil spring (.010"×0.030", FDA ,USA) were compressed between these bands along the rectangular stainless steel wire 0.016"×0.022" (Dentarum,Germany)to produce a 60g of reciprocal force measured with a dynamometer (Hahnkolf,Stuttgart, Germany) ,this wire was inserted into band slot with ligature elastic (Dentarum,Germany) and was then crimped over on the ends to prevent its loss as shown in Figure(1) . The springs were not reactivated during the experiment

▪ Radiographic Examination:

Standardized digital radiographs using a planmeca Dimaxis Pro X-ray machin with dimaxis classic imaging software (Helsinki,Finland 2003); were taken after sacrifice. The mandible of the animal was placed in contact with the sensor and the inferior border of the mandible is parallel to the sensor and at a constant distance (the distance between the end of the long cone and the sensor was fixed to 5 cm) and the cone was kept perpendicular to the sensor all the time. These radiographic images were taken at 60KVp,8mA and .048sec and the read out starts automatically, the image was displayed gradually on the computer screen, when the read out was completed, the newly read image was stored.

▪ Measurement of tooth movement:

The amount of tooth movement was determined by measuring the distance of tooth movement in millimeters every morning between the midpoint of the narrowest space between mandibular central incisors at the level of alveolar crest and mesial surface of root for right and left mandibular incisors on the occlusal radiograph using digital radiograph . Readings were taken over the 22 day experiment at 1, 2and 3 week under the same anesthesia protocol . The tooth movement velocities (millimeters per week) was calculated as the amount of tooth movement divided by the time period. To standaradize the amplification ,to increase the accuracy of the small tooth movement measurement changes,the images were magnified two times the original size with the known scale and were traced with selected landmarks and distance.

- **Measurement of bone density**

A standardized grid was superimposed to each figure using Image J(Natural institute of health USA software). The grid was set up so that each millimeter would have approximately 4 squares. Then the bone density was measured on the tension side of both right and left root of mandibular central incisor at 0.5mm interval from 0.5 to 1mm proximal to the mandibular symphysis at right and left side and at 1-mm intervals from 0 to 8 mm moving apically from the alveolar crest on the coronal plane (Figure 2). Therefore the total measurements were made by 36 sites , which is the area of interest using image j. By assigning the value (0) to black and (256) to white, the mean gray value in each region of interest was calculated in pixels. Areas of bone loss represented as darker areas, while areas of bone gain represented as lighter areas according to Yokota et al.[(19)] The measured bone density values were averaged for the subjects, keeping specific to the designated area divided by cervical (0–2 mm), middle (3–5 mm), and apical (6–8 mm) as well as medial(0.5 mm) and lateral(1 mm) segment. The bone density was measured at the 1st ,2nd and 3rd week of incisors distalization.

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM SPSS Statistics, Version 19 (IBM Corporation, USA) . Descriptive statistics were given as mean and standard deviation.To compare means of tooth movement and bone density between two sides, independent samples T-test was used. One-way ANOVA was used to compare means of more than two sides, followed by Duncan's multiple range analysis test.

RESULTS

The comparison of the amount and velocity of tooth movement between control and 1,25 DHCC sides are illustrated in Table(1). The amount of tooth movement was greater in 1,25 DHCC side when compared with the control side at all time intervals .The amount of tooth movement increased with time in both sides particularly in the 1,25 DHCC side ,while the velocity of orthodontic tooth movement decreased with time in both sides as shown in Table (2) and Figure(3). The descriptive statistics of bone density for the control and 1,25 DHCC side in 1st ,2nd and 3rd week are shown in Table (3) Comparison of bone density between both sides (Table 4) showed no significant difference in all sites and at all time intervals .However the 1,25 DHCC side had higher bone density than the control side as illustrated in Figure(4) The medial bone density was higher in the middle area on the 1st and 2nd weeks in both sides when compared with the lateral bone density .In addition, there was increased medial bone density in the cervical region at right side and in the apical region at the left side. In comparison, on the 3rd week the medial bone density was higher than lateral in the apical area at both side (Table 5).

One way ANOVA showed there are significant differences of medial and lateral bone densities among different areas for both sides (Table 6) where the apical area showed the highest density and the cervical area showed the lowest density (Figure 5) Comparison of bone density among different weeks on both sides showed significant differences in the middle and apical area (Table 7). On the other hand, the lateral bone density showed significant difference at cervical region only. Duncan multiple range test showed that medial bone density was higher at 3rd week in all areas and that lateral bone density was higher at 3rd week in the cervical area for both sides(Figure 6)

DISCUSSION

Animal Species

All previous experimental studies were carried out on rats, mice and very few in rabbits. In this study albino rabbits were used because of ease of handling and fabrication of intraoral appliance is comfortable compared to rats and also similarities are reported in the bone mineral density (BMD) and fracture toughness.[(17)].

Study design

Mandibular rather than maxillary central incisors were selected to fix the orthodontic appliance because of their greater cervico-incisal length and better accessibility for preparation and placement of the appliance [18]. A randomly assigned incomplete block split-mouth design was used for this experiment to reduce inter individual variation. The rational behind choosing these doses is corresponding to previous reports about 1,25 DHCC, which showed its influence on bone remodeling and found to be dose dependent and effective locally within the minimum normal physiological dose [20]

Differences of OTM between control and 1,25 DHCC side

The comparisons of the velocity of central incisor movement between the control and 1,25 DHCC side at each interval showed that the 1,25 DHCC side had greater amount and velocity of tooth movement as compared to control side. This finding comes in agreement with previous animal studies, which indicated that the effect of 1,25 DHCC on OTM is highest when administered in doses relatively equivalent to the normal physiologic level[6,7,20]

Differences of OTM among different time intervals in both sides

It is generally considered that orthodontic tooth movement consists of clearly separatable three phases occurring in the periodontal ligament: an initial compressive phase consisting of changes in the viscoelasticity of periodontal ligament for 1-4 d; a follow-up lag phase in which the tooth movement slows down for 4-7d with the appearance of tissue with no cells and fibers in the periodontal ligament; and finally a phase in which the tooth moves progressively in association with bone resorption for 7-14d [21].

Both sides showed similar patterns of increase or decrease in the velocity of central movement per the three intervals, The greatest velocity of tooth movement was on the first week in both sides .However the velocity of orthodontic tooth movement in the 1,25 DHCC side was higher than the other side indicating that 1,25 DHCC can accelerate orthodontic tooth movement as the result of a reduction of the initial lag phase when force is applied.

Comparison of bone density between control and experimental sides

In the present study, radiographic analysis was carried out for evaluation of bone density using direct digital system that has many advantages, among which, ability of image adjustment and manipulation , possibility of image storage and the stored images can be used almost instantaneously[22,23] Both sides showed similar patterns of increase or decrease in the bone density, indicating the presence of mild and non significant systemic influence of 1,25- dihydroxycholecalciferol which led the control side to follow the general pattern of the 1,25 DHCC side,however the bone density was higher in 1,25 DHCC side compared to the control side at all intervals, this indicates the ability of locally injected 1,25 DHCC on reducing treatment time and cost not only at the right side but at the control side also (but to a lesser extent). The medial bone density was higher than lateral bone density and the main reason for this could be that the newer bone induced by the application of orthodontic forces having lower mineralization and being less dense than older bone [24].

In general, there was a progressive increase in bone density from the cervical (lower density) to the apical region (higher density) this is in accordance with Verna et al. [25] who found variations in the changes in different tooth portions, but in contrast to Bednar etal [26] who found the mean bone-density changes around each tooth did not differ significantly among the cervical, intermediate, and apical portions after orthodontic treatment. Such differences, if actually present, might be caused by tooth movement with sliding occurring in a stepwise manner involving tooth tipping and uprighting rather than as a continuous sliding process Generally, the bone density increased with time, this may be attributed to alveolar bone may experience a transient decrease of bone density while resorption predominates earlier, followed by a return to normal density while bone formation predominates.

In addition, continuous mechanical stimuli can lead to increased bone density, a phenomenon known as SATMU (Structural Adaptation To Mechanical Usage) [27] Therefore, the decreased alveolar bone density observed in some studies may be due to the short interval between the start of the application of orthodontic force and the measurement, while a longer interval between the events will allow more time for bone formation to occur and result in higher alveolar bone density to be observed

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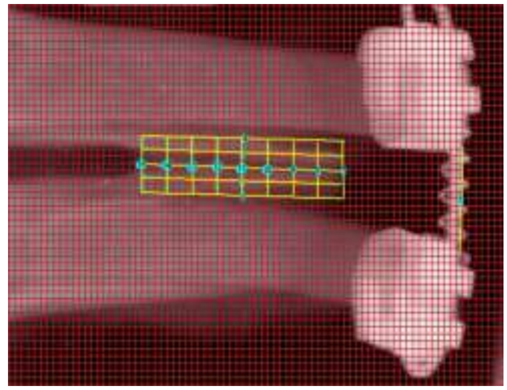

Figure (1):Orthodontic appliance in situ. **Figure (2):Radiographical measurement of bone density.**

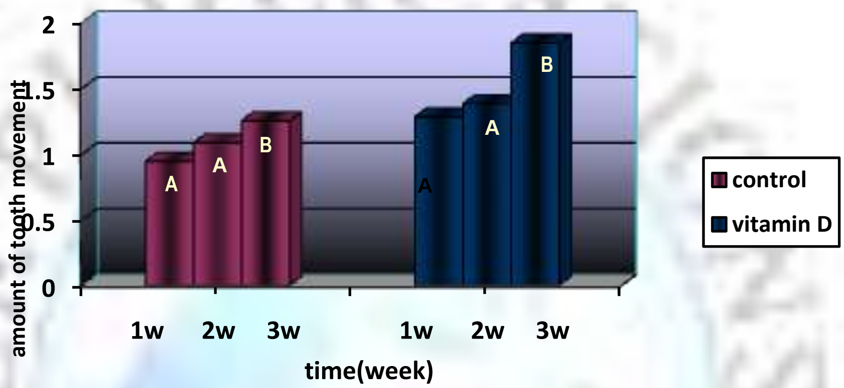

Figure (3): Comparison of amount of tooth movement among different weeks in the control and 1,25 DHCC sides.

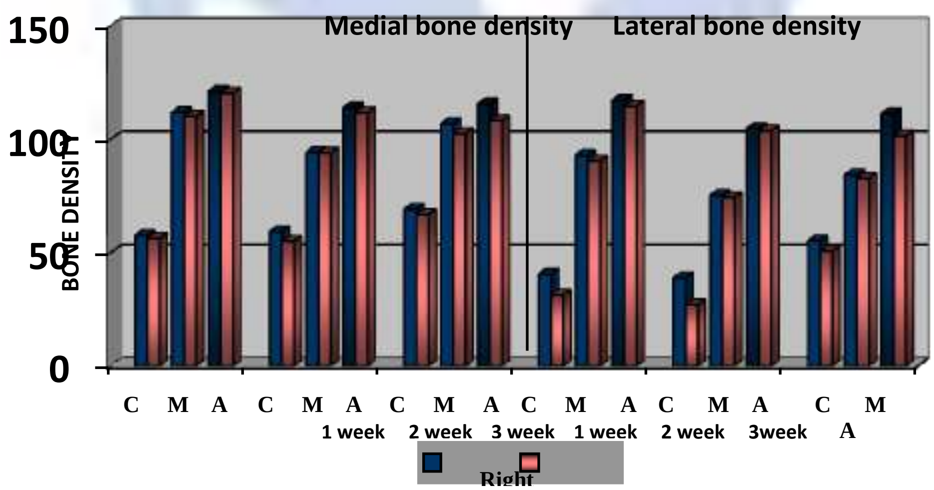

Figure (4): Comparison of medial and lateral bone density between control and 1,25 DHCC sides at different times
(C=cervical, M=middle, A=apical)

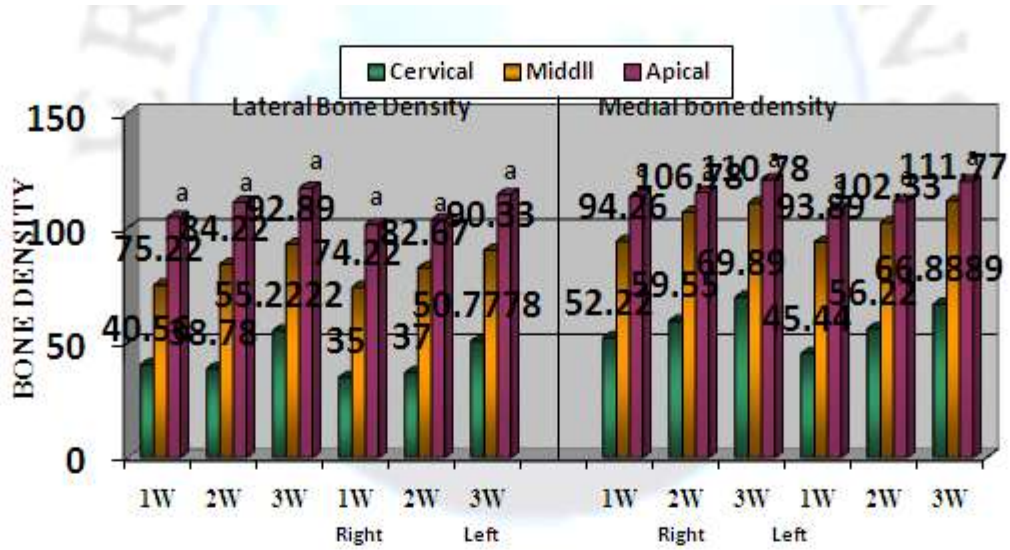

Figure (5): Comparison of lateral and medial bone density among different sites at both sides on different time intervals. *Different letters for each week mean significant difference.

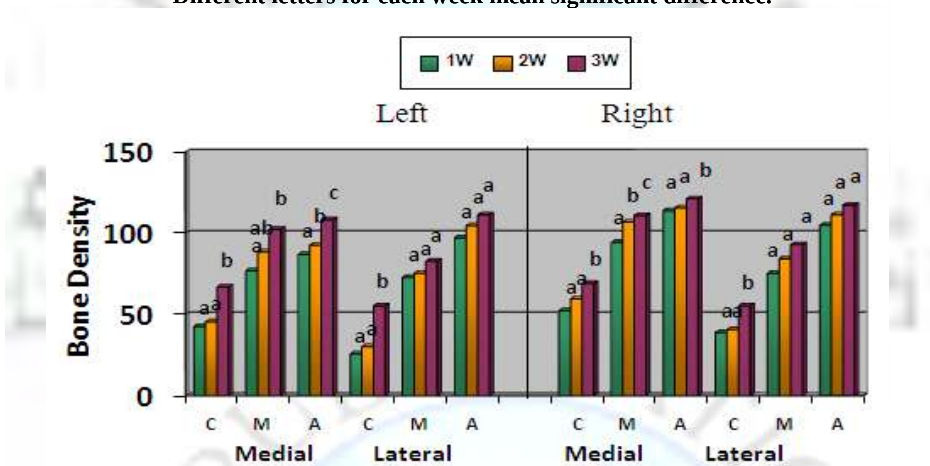

Figure (6): comparison of medial and lateral bone density among different weeks at both sides . (C=cervical, M=middle, A=apical)

*Different letters for each site mean significant difference.

Table (1): Comparison of amount and velocity of tooth movement between control and 1,25 DHCC side at 1,2,and 3 week.

Week	Side	Amount of OTM		Velocity of OTM		T value	Sig.
		Mean	SD	Mean	SD		
1	Control	.940	.219	.948	.219	2.666	.056*
	1,25 DHCC	1.280	.026	1.280	.026		
2	Control	1.083	.121	.542	.060	3.102	.036*
	1,25 DHCC	1.380	.114	.693	.055		
3	Control	1.250	.145	.403	.045	5.552	.006*
	1,25 DHCC	1.840	.142	.613	.047		

* indicates significant values, ($p < 0.05$)

Table (2): Comparison of the amount and velocity of tooth movement among different weeks in both sides.

Side	Week	Amount of OTM	Velocity of OTM	Duncan	F- value	Sig.
		Mean	Mean			
Control	1	.940	.948	A	12.996	.007*
	2	1.083	.542	A		
	3	1.210	.403	B		
1,25 DHCC	1	1.280	1.280	A	199.866	.000*
	2	1.380	.693	A		
	3	1.840	.613	B		

* indicates significant values, ($p < 0.05$)

Table (3): Descriptive statistics of bone density in the control and 1,25 DHCC side at different time intervals .

Position		Side	1 Week		2 Week		3Week	
			Mean	SD	Mean	SD	Mean	SD
Medial	cervical	control	42.500	28.519	45.333	26.650	66.888	17.215
		1,25 DHCC	43.000	25.796	69.111	19.009	70.600	9.545
	middle	control	75.500	16.935	88.466	9.432	102.333	8.930
		1,25 DHCC	76.916	22.912	93.266	6.943	106.777	12.930
	apical	control	77.636	10.709	92.600	26.607	108.222	11.475
		1,25 DHCC	86.909	19.304	96.733	9.728	115.555	5.270
Lateral	cervical	control	25.133	16.492	30.333	18.058	50.777	14.245
		1,25 DHCC	28.666	17.916	32.200	21.014	55.222	12.346
	middle	control	72.200	14.212	73.333	12.633	81.222	11.144
		1,25 DHCC	74.777	16.230	81.000	16.795	84.666	5.937
	apical	control	97.111	7.573	102.80	7.974	103.333	6.576
		1,25 DHCC	101.888	12.251	103.73	11.578	111.222	13.414

Table (4): Comparison of medial and lateral bone density between control and 1,25 DHCC side at cervical ,middle and apical site at different weeks.

medial bone density									
Week	1 week			2 week			3 week		
Site	Ant.	mid	post	Ant.	Mid	post	Ant.	mid	Post
T value	.045	-.172	.802	.802	1.587	1.175	.260	.848	1.742
Sig.	.964	.865	.179	.429	.124	.250	.798	.409	.101
Lateral bone density									
Week	1 week			2 week			3 week		

Site	Ant.	mid	post	Ant.	Mid	post	Ant.	mid	Post
T value	.698	1.421	.995	-.429	.160	.257	-.707	.370	1.584
Sig.	.495	.174	.334	.671	.874	.799	.490	.717	.133

Table (5): Comparison between medial and lateral bone density at both sides in cervical ,middle and apical site at different weeks.

	1,25 DHCC side								
Week	1 week			2 week			3 week		
Site	Ant.	mid	Post	Ant.	Mid	post	Ant.	mid	Post
T value	2.315	3.964	.902	2.981	5.123	-1.793	1.183	-1.073-	-3.529
Sig.	1.652	.001	.380	.007	.000	.084	.251	.297	.002
	Control side								
Week	1 week			2 week			3 week		
Site	Ant.	mid	Post	Ant.	Mid	post	Ant.	mid	Post
T value	1.652	5.502	1.109	1.499	3.043	-3.176	1.551	.461	-2.619-
Sig.	.120	.000	.284	.145	.005	.004	.137	.650	.017

* indicates significant values, ($p < 0.05$)

Table (6):Comparison bone density among different sites on the1 st ,2 nd and 3 rd week in both sides.

	Medial bone density					
	1,25 DHCC			Control		
	1 week	2 week	3 week	1 week	2 week	3 week
F- value	16.669	34.084	29.547	8.266	37.750	26.580
Significancy	.000	.000	.000	.001	.000	.000
	Lateral bone density					
	1,25 DHCC			Control		
	1 week	2 week	3 week	1 week	2 week	3 week
F- value	67.787	108.364	48.818	48.494	76.206	68.101
Significancy	.000	.000	.000	.000	.000	.000

*
indicates significant values, ($p < 0.05$)

Table (7): Comparison of medial and lateral bone density among different weeks at both sides in different site.

	Medial bone density					
	Right			Left		
	cervical	middle	Apical	cervical	middle	Apical
F- value	2.942	16.597	24.603	3.002	3.866	12.268
Significancy	.067	.021	.000	.063	.031	.000
	Lateral bone density					
	Right			Left		
	cervical	middle	Apical	cervical	middle	apical
F- value	5.894	2.265	1.816	7.206	1.44	1.407
Significancy	.007	.121	.180	.003	.259	.261

* indicates significant values, ($p < 0.05$)