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Research Article

Effect of Local Administration of Clodronate on Bone Density After Orthodontic Treatment

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Abstract

Aim: The aim of this research was to evaluate the local effect of clodronate on created space done by orthodontic teeth movement on rabbits teeth.

Materials and Methods: A twenty local rabbits of age 6 months to two years, their weight about 1,25 - 1,75 Kg. The rabbits grouped into control, and experimental group with sub-grouping into 2 and 3-weeks, 5 rabbits for each group. The appliance fixed on lower incisors of the animal. Clodronate is drug that given locally at the mesial side of incisors. Testing of the animals were done by body-dual-energy x-ray densitometer, tissue assessment for the number of osteoblast and osteoclast for all the groups with hematoxylin and eosin stain.

Results: High mean value in bone density test for experimental group, with significant difference found between it and control groups of same period. Cell numbers (osteoblast) are higher in 2nd than 3rd week period for both groups, and higher value found in experimental group than control one. A non-significant differences for osteoclast number found among the sub-groups.

Conclusion: Local clodronate is effective in reducing the unwanted tooth movements by increasing the density of bone thus reduce relapse. Best advantage of clodronate was seen in third week of retention after drug withdrawal.

Keywords: *Local Administration; Clodronate; Bone Density; DXA; Relapse*

Introduction

One-of the challenging-troubles facing the orthodontists and patients is relapse. Orthodontic relapse has been known as "The loss of any improvement accomplished by orthodontic treatment" [1,2].

The retention-phase was elementary for insistence of orthodontic treatment so as to maintain teeth at a correct place after ending of teeth movement [3]. "Pharmacological anchorage method," is alternate advance which is, local given of drugs specially, Bisphosphonate close to the teeth were planned in order to make resistance against-arrangements [4,5]. Bone-renewal is a-guide in orthodontic-application where it has been used for different purposes, mainly as anchorage [6].

Bisphosphonates, for instance clodronate-($CH_2Cl_2O_6P_2Na_2 4H_2O$), are drugs which hold down osteoclasts in bone and lessen bone turnover. Clodronate has been showing to stop or retard skeletal-related actions and lessen bone-pain and so regularize calcium levels [7].

The anabolic action of anti-resorpative drug to enhance bone formation is a good method to increase bone mass. Clodronate are Bisphosphonate lacking amino groups that improve vertebral density and sustain femoral neck density. The use of bisphosphonates that used for the treatment of periodontitis were established in animal, Alendronate was revealed to restrain bone defeat about affected teeth in contrast to controls [8]. The capability of clodronate to affect bone osteogenesis when given systemically elevate the question, whether local effect of Clodronate has the same effect as systemically. Alveolar bone loss (ABL) in periodontitis have regular mechanism. General bone conditions effect on the periodontium. Bisphosphonates increase bone mineral density is accounted to be connected to (ABL) and to clinical connection loss. Bisphosphonates sluggish the resorption of inter dental bone of the maxilla and mandible also to improve periodontal condition [7].

Clodronate Liposome is efficient in reducing macrophages, when used either systemically or locally [9]. Bone minerals density (BMD) is a sign of bone strength and potency. This is a golden method for formative hazard of prospect of fracture. The measurement is supported by dual-energy X-ray absorption (DEXA), and densitogram analyses. Using dual energy (DXA) lessen the possible for wrongness happened by soft tissue. DXA specify the consequence as surface is a density (g/cm^2) [10,11].

Bone mass is low at birth and raises for the next two - three decades as the action of bone-forming osteoblasts exceed that of the bone-resorbing osteoclasts, eventually leading to peak bone density through young adulthood [12].

Aims of the Research

It was undertaken to evaluate the local effect of clodronate on created space done by orthodontic teeth movement on rabbits teeth, at retention in order to reduce retention period and lessen the relapse. Bone density will be evaluated by densiometric analysis DXA. Also effects of clodronate on bone structure by histological examination.

Null hypothesis

There is no difference in bone density between experimental and control groups.

Alternate hypothesis

There is difference in bone density and tissue response to drug administration for experimental group than the control one.

Materials and Methods

This research was done in Dentistry College, university of Mosul. The approval for this work, obtained from the college of dentistry ethics committee (Protocol ref no. 1548).

Twenty local females rabbits of age 6 months to two years, weight about 1,25 - 1,75 Kg. The rabbits were housed with normal conditions of good ventilation, sufficient diet, and they had free access to tap water. Animals were separated into two groups (control and experimental group) subgroup, 2-weeks and 3-weeks, 5 rabbits for each group. Drug used is Clodronate, disodium Clodronate in trade name (CLODRONATO ABC).

Intra-muscular injection of ketamine (10 mg/kg), with diazepam (5 mg/kg) Bw was given to the rabbit, then the appliance fixed on lower incisors which is made of two stainless steel bands for mandibular incisors teeth, size 0 consecutively to fit to rabbits teeth. A stainless steel arch wire 0.016”×0.022” used for movement of teeth. Open coil spring (Nickel Titanium) of 5 circles and 5 mm in length to exert 50 gm force on the two teeth, the length of the spring selected to deliver the amount of force that needed to separate the incisors when the spring activated. 0.01” ligature wire used for ligation the brackets band to the arch wire (Figure 1), then allowing the appliance to be active, teeth distalization were completed during two weeks. Another week for completing any teeth movements and for initial retention,

at this point the periods of groups were determined into 2 and 3 weeks, and administration of drug for the experimental groups. Injection of Clodronate 0.3 mg/kg body weight given locally at the mesial side of mandibular central incisors in the mucoperiosteum region [4].

Figure 1: *The appliance fixed into rabbits teeth.*

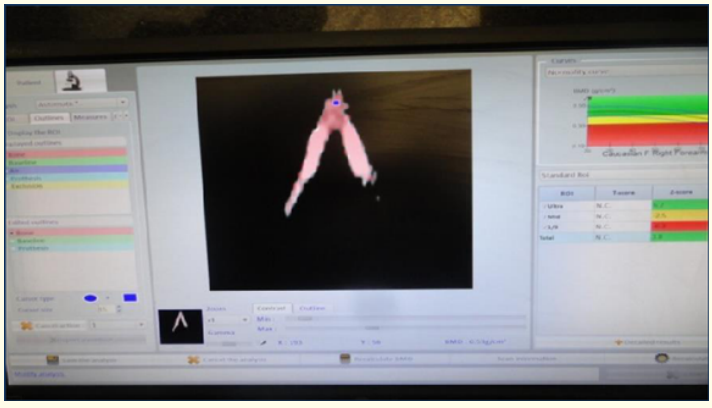

Figure 2: *Software used for DXA scan.*

Figure 3: *DXA device.*

Measuring bone density

Scan of the samples done using body dual-energy x-ray densitometer (DEXA- STRATOS, UK) with software (Nero, version 5.5.10). The field in between the teeth with specific area, this make the researcher to know the density of any size, in any position on the scan. Values of the bone mineral density (BMD) were in units of g/cm^2.

The histological testing

Decalcified bone slices and slide were prepared and staining it with hematoxylin and eosin (H&E) stain, histological examination for the number of active osteoblast for all the groups. The number of active osteoblast were counted, scoring of osteoclast were + (1-10), ++ (11-20), or +++ (>20) [13].

Statistical analysis

Data will be processed and analyzed using SPSS (statistical package for social science). P-value ≤ 0.05 will be considered as significant. The descriptive analysis for all the variables, independent t-test used for comparison between two groups, z-test and Mann-Whitney analysis used for non-parametric variables.

Results

Localized bone density

Table 1 represent description of bone density in between incisors. The highest mean value seen in the 3rd week Clodronate group, while the lowest mean value seen in control 2nd week group.

Variables	Minimum	Maximum	Mean	SD
Cont. 2 week	0.40	0.43	0.416	.0133
Cont. 3 week	0.42	0.50	0.453	.0352
Exp. 2 week	0.55	0.64	0.569	.0388
Exp. 3 week	0.55	0.64	0.596	.0412

Table 1: *Description of localized bone density.*

In table 2 we notice the comparison between each two groups by independent t-test, a significant difference can be found between clodronate and control groups of same period. Non significant difference found within the same group, that clearly seen in figure 4.

Variables	Mean	SD	t value	Sig.
Cont. 2 w	0.416	0.0133	2.760	.070
Cont. 3 w	0.453	0.0352		NS
Exp. 2 w	0.569	0.0388	1.387	.259
Exp. 3 w	0.596	0.0412		NS
Cont 2 w	0.416	0.0133	6.598	.007
Exp. 2 w	0.569	0.0388		S
Cont 3 w	0.453	0.0352	5.744	.010
Exp. 3 w	0.596	0.0412		S

Table 2: *Comparison between each two groups.*

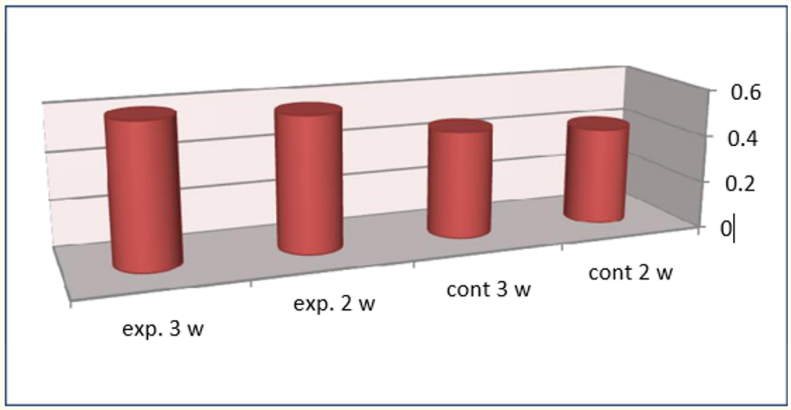

Figure 4: *Mean values of all the group.*

Histology

Measurements done by x4 of the microscopic lens. For untreated group a tissue in between mandibular teeth seen in figure 5A that the width at upper (cervical) area is 113µ. In the middle part the width is 149.5µ. At the apical part the width is 176µ. The periodontal ligaments width is 14.5µ for all the parts. The width of area in between incisors for all the groups, started at the periodontal ligaments in mesial surface of right tooth to the periodontal ligaments in mesial surface of left tooth. A x4 power section, cervical area dimension was 245µ, Middle was 218µ and at apical part is 208µ, the mean value width of periodontal ligaments 18µ. There was a smooth root continuity surface, there is no resorption for the experimental groups, can be seen in figure 5B.

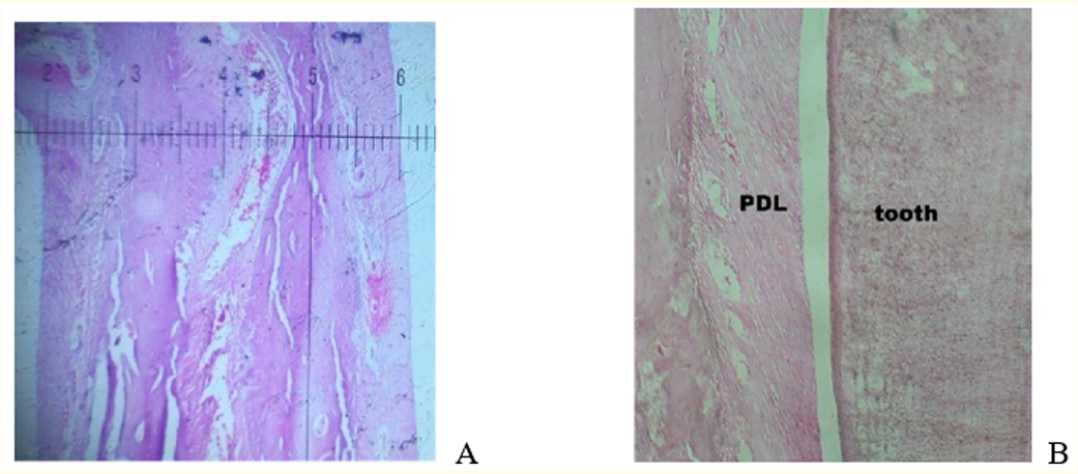

Figure 5: *Histological section (x4), A- Width of alveolar area for untreated group, B- Root surface (no resorption) in experimental group. H&E stain.*

The area of osteoid tissue in figure 6 was detected in the cervical parts of rabbit's tooth seems to be larger than in the middle or apical parts, that is why an estimation of osteoblast and osteoclast number done in the cervical area only. Osteoblast cell numbers are higher in 2nd than 3rd week period for both groups, and higher value found in experimental group than control one, this clearly seen in figure 6 and 7.

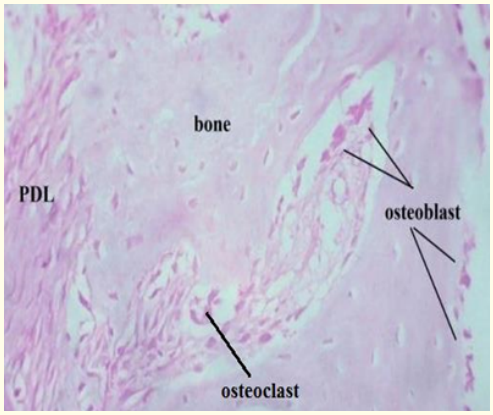

Figure 6: *New bone formation with haematoxyline and eosin staining in experimental group (magnification 10x).*

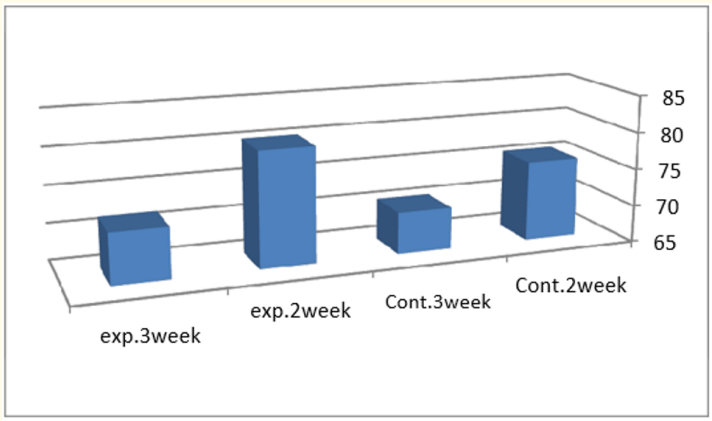

Figure 7: *Represents mean value of osteoblast among the groups.*

The highest mean of osteoblasts number shown in figure 7, for 2nd experimental group, followed by 2nd control, then the 3rd experimental group, and finally the 3rd experimental one has the lowest mean value. For comparison the number of osteoblast among the groups in table 3, in-depended t-test shows; a significant differences between 2nd and 3rd week for control and experimental groups. Also significant differences between control and experimental groups in the 2nd week period, while no significant differences between control and experimental groups in the 3rd week period. In table 4 Z test used for comparison of osteoclast number, shows anon significant differences between the periods within the same group. Also no significant differences between the groups (experimental and control) within the same periods can be seen in table 5 using Mann-Whitney test.

Variables	Mean	SD	t value	Sig.
Cont. 2 w	75.80	1.48	5.20	0.001
Cont. 3 w	70.60	1.67		S
Exp. 2 w	80.40	1.67	5.13	0.002
Exp. 3 w	71.80	3.34		S
Cont 2 w	75.80	1.48	7.88	0.001
Exp. 2 w	80.40	1.67		S
Cont 3 w	70.60	1.67	5.88	0.501
Exp. 3 w	71.80	3.34		NS

Table 3: *Comparison the number of osteoblast between each two groups.*

NS-Non Significant, S-Significant.

	+	++	+++	Z test	P value
Cont 2 week	0	6	0	0.01	0.98
Cont 3 week	6	0	0		NS
exp 2 week	5	0	0	0.44	0.65
exp 3 week	0	2	0		NS

Table 4: *Comparison the number of osteoclast of different periods within the same group.*

	Mann-Whitney U	P value
Cont 2 week	4.00	0.796
exp 2week		NS
Cont 3week	4.00	0.796
exp 3week		NS

Table 5: *Comparison numbers of osteoclast for different group within the same periods.*

Discussion

In this research; the results established that the use of drugs that positively effect on bone regeneration may be benefit to increases teeth retention and reducing relapse, although it is not a wise clinically to use local injection of all dental arch, but the more area susceptible to undergo the unwanted movement like canine tooth.

An increase in bone density that was seen in experimental groups for both periods (2^{nd} and 3^{rd} week), indicate the effect of local injection of Clodronate, which could be due to the anti-resorptive activity (anti-osteoclastic action).The significant increase of bone density was markedly reduced the relapse of incisors in mesial direction after withdrawal of drug also support the topical effect of clodronate. This comes with agreement of Liu., *et al*. [14] and Venkataramana [4], who used Ibandronate to reduce tooth movements Al-Sultan [15], proved a significant raise in bone density and bone structure take place with local alendronate.

Bone density test (which is a precise method to know the density of bone in specific area), made at area which undergoes changes; spacing between teeth and changing of bone density (decreases as teeth moved distally then increases at retention period, this clearly seen that the density of 2nd week less than that of 3rd week period for both groups. this agreed with Haderslev., *et al*. and Tanakol., *et al*. [16,17] they found that systemic Clodronate administration for prevention of osteoporosis, increases the density of new bone formation. This demonstrated the inhibition of relapse in experimental group.

Tapaninen [18] proved that there is advantage from Clodronate administration for rising bone mineral density by DXA scanning of the bone in arthroplastic operation for females. Lucisano., *et al*. [19] found that systemic Bps(alendronate) treatment revealed increases of bone mineral density in rats, as noticed radio graphically and by DXA, in that; both of techniques were capable to identify enhancement in bone mineral density. The results of Pennypacker., *et al*. [20] explain that, systemic management of alendronate will raise femur bone mineral density in rabbits calculated with DXA.

These results proved that topical treatment of Clodronate could be efficient in stopping osteoclastic resorption in periodontitis as demonstrated by Mitsuta., *et al*. [21] and by Jakobsen., *et al*. [22] whose, there experimental studies specify that the use of bisphosphonates can improve bone value in animals and lead to a 1.3-fold increase in bone volume around implants following joint alternate surgeries.

For osteoblast activity, which were calculated at cervical part of the root; since it is wider than middle and apical part. We found a maximum number of cells (98) seen in the 2nd week period of control group, while a minimum number of cells (33) seen in control group 3rd week period than others. These differences related to the variability among the animals. In other hand a larger mean value (86) found in the 2nd week period of experimental group, and a smallest mean value of osteoblast (70) found in control group 3rd week period. So supplementation with Clodronate histologically increasing fibroblasts count following orthodontic retention period. Xiong., *et al*. [23] establish that Bisphosphonates (etidronate) encourage the differentiation of rat osteoblasts. Little., *et al*. [24] found that pamidronate demonstrated an enhance in osteoblastic rimming and mineralization of the re-developing, improvement the configuration of bone around the pin sites and a raise in the cortical width of the bone close to the regenerate in the rabbits.

A significant difference present between experimental and control groups for both periods, this agreed with Krishnan., *et al*. [25] who said that, Bps (alendronate) shows a significant increase in the number of osteoblasts and a significant reduce in the number of osteoclasts in both the pin and the regeneration regions. These results showed significant enhancement in bone remodeling through osteogenic distraction, which causes enhance in callus formation.

The study of D'Aoust [26] and Abid [27] proved that; bisphosphonate treatment promotes osteoblastic delineation and Alendronate encourage osteoblast precursors and mineralized nodules, thereby promoting early osteoblasto-genesis. Where Rosenqvist [7] said that; bisphosphonate had indirect belongings on osteoblasts. *In vitro* facts proposed that bisphosphonate reduce osteoclast employment and continued existence by performing the cells of osteoblast lining, and additionally induct osteoblast action to emit the inhibitor for osteoclast employment and survival.

Ryabov [28] found that bisphosphonates improve the action of bone morphogenetic proteins and excite configuration of new bone. While Saarto., *et al*. [29] said Clodronate management the previous HRT still had a significant advancing the effect on BMD changes particularly in lumbar spine.

Kirkwood., *et al*. [30] said that the activity of osteoclast is reserved as Clodronate introduced in the bone; the author proved that bisphosphonate influence osteoclasts in direct and indirect way. A direct relation of osteoclasts and/or osteoblasts rivet a diversity of biochemical path (like receptor-activator of NF-Kappa B, changing the growth factor and bone morphogenic proteins mediated trail). Clodronate also has direct stimulate tumor cell and osteoclast apoptosis and decreases cell bond and attack in pre-clinical molds. More-

over, Rosenqvist [7] found that bisphosphonate have an indirect consequence via osteoblasts. An *in vitro* confirmation proposes that bisphosphonate slow down osteoclast employment and survival by working on cells of osteoblast line. In that bisphosphonate minimize osteoblasts capacity to create osteoclast provoking agent and more significantly induct osteoblastic activity to exude the inhibitor for osteoclast employment and survival. Bisphosphonates sluggish the resorption of alveolar-bone of maxilla and mandible in addition it have been showed to progress periodontal status as stated by Jeffcoat [31].

Conclusion

This study proves the use of local clodronate is effective in reducing the unwanted tooth movements by increasing the density of bone thus reduce relapse. Best advantage of clodronate was seen in third week of retention after drug withdrawal.

Ethics Approval and Consent to Participate

The approval for this work, obtained from the college of dentistry ethics committee (Protocol ref no. 1548).

Conflict of Interest

There is no conflict of interest.

Funding Support

No funding for this work.

Consent for Publication

On behalf of all the contributors I will act and guarantor and will correspond with the journal from this point onward.

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Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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