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ROLE OF LOCAL INJECTION OF 1, 25 DIHYDROXYCHOLECALCIFEROL IN PREVENTION OF POST ORTHODONTIC RELAPSE IN UREMIC RABBITS

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Abstract:

Objective: *this study aimed to evaluate the effect of local injection of vitamin D 1, 25 Dihydroxycholecalciferol (VD) by means of measuring OTM and bone density by computerized tomography the number of osteoclasts during orthodontic relapse in uremic New Zealand rabbits after induced nephropathy.*

Materials and Methods: *This study is in vivo experimental research. A total of 34 male New Zealand rabbits were used in the study, which were randomly split into three groups, control group (CG), uremic group(CKD G) and uremic with locally injected VD (CKD with VD G) . All rabbits were given an orthodontic force of 60 cN using a NITI 3-spin coil spring that was activated for 15-18 days and then conditioned to be passive. Through this phase, the CKD with VD group was locally injected with VD once every week for 4 weeks. CT measurements and histological analysis were formed at 18 days, after removal of the Orthodontic appliances and after 38 days, Data were analyzed statistically using an independent t-test ($p < 0.05$).*

Results: *The post OTRM was significantly lower in the CKD with VD injection than in the CKD ($P=0.01$). The number of osteoclasts in the CKD with VD group was significantly lower than that in the CKD group ($p = 0.021$).*

Conclusion: *locally injected VD can prevent post orthodontic tooth relapse movement by suppressing osteoclastogenesis and decreasing osteoclasts number in uremic rabbits.*

Introduction:

Orthodontic treatment is a process that corrects the teeth to gain valid normal occlusion function[(1)]. During orthodontic procedures, the ultimate issue confronted by an orthodontist is relapse, which results from changeful bone remodeling process. Bone remodeling is a complex persistent process of bone destruction and renovation [(2)]. During orthodontic relapse Osteoclast formation, known as osteoclastogenesis is increased [(3)].

Suppression of orthodontic relapse is tried in several ways as pharmacologically by using drugs such as bisphosphonates and mechanically by using a retainer. However, both of them induce other effects in the patients [(4, 5)]. In chronic kidney disease, bone remodeling is compromised. Furthermore, Uremia is considered as a metabolic bone disturbance affecting the manipulative proceedings such as orthodontic procedures [(6)]. Several studies on orthodontics have found that 1, 25(OH)2 dihydroxycholecalciferol (Vit.D3) injection decrease formalization of osteoclasts and thus decrease bone resorption [(6, 17)]. Knowing that Vit.D3 affects bone metabolism in an anabolic manner, osteoblastic cells have high-affinity receptors for Vit.D3, a finding that strengthens the idea of its regulatory bone formation and mineralization effect.

The hypothesis of this study was that locally

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injected vitamin D could suppress post orthodontic relapse by reducing osteoclastogenesis and decreasing osteoclasts number.

Materials and Methods:

Animals: The experiment included 34 New Zealand healthy male rabbits, ranged from 2.5-3 kg in weight and 10-11 months in age at the start of the study. Rabbits were maintained in aerated room on a natural 12:12-h dark-light cycle, temperature (25 ± 5°C), standard meals and water were available. Animals were habituated for handling and adapted to laboratory conditions for one week before the start of the experiment. Animals were randomly assigned into three groups: Control healthy group (n =10), in which only OTM was conducted. Uremic group with no VD injection (CKD group) (n=12), in which nephropathy was induced by gentamycin, and uremic group with VD injection (CKD with locally injected VD) (n=12), which were treated by local injection of Vit. D3 after nephropathy induction and then orthodontic procedures was done in the three groups

Induction of nephropathy in rabbits: Aminoglycosides (AGE) (AGE-treated groups) were injected intraperitoneal in dose of 150 mg/kg/day. Uremia happened within 10 days. After that, a maintenance dose of 20 mg/kg/day is continued to preserve the uremic state [(7)].

Application of orthodontic appliances: After uremia occurrence with ten days, the rabbits were anesthetized with a xylazine hydrochloride 23.3mg/mL (Xyla-ject) and ketamine hydrochloride 50 mg/ mL (Ketamine) intramuscularly. The appliance was inserted for all groups. Appliances were similar to those of **Abtahi et al**. [(8)]. A nickel-titanium closed-coil spring appliance was ligated between central incisor and mandibular right first premolar with a 60 g force and was activated for 18 days and then conditioned to be passive.

Measurement of tooth movement and bone density: Measurements were obtained twice; first measurement: After activation of the appliance for 18 days and its removal, and the second after appliance removal with 20 days. Mesiodistal space between the mesial surface of the mandibular right first premolar crown and the distal surfaces of the mandibular right central incisor crown was measured by computerized tomography (CT).

Method and Dose of Vit.D3 administration: A locally injection of twenty micro liters of 10-10 mol/L; 1, 25(OH) 2D3; in the sub mucosa distal and mesial to the apical root of right premolar, once per week. Rabbits were injected on days 14, 21, 28, and 35 day .

Positioning of the appliance: to move the right mandibular incisor distally against mandibular first premolars.

Histological analysis: was formed twice, first analysis where the specimen was obtained by true cut fine needle biopsy at 18 days and the last one at the end of the study from the rabbit's lower jaw, fixed in neutral buffer formalin 10% for one week. After fixation, decalcified samples were washed to remove formic acid, embedded in paraffin wax. Serial sections were gained and stained with hematoxylin and eosin (H and E) as well as Masson trichrome stains assembling Garcia-Donas et al. protocol [(9)]. For electron microscopy, decalcified samples analyzed in JEM 100 CX11 transemission microscopy (TEM) then processed in the Electron Microscope Unit of Assiut University; Photographs were taken by digital camera Model XR-41M linked to a DELL desktop computer [(10)].

Statistical Analysis: Statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA) was used. Parametric tests were used to analyze the data. Frequency and percentage were used to express Qualitative Datum while Quantitative Datum was expressed as mean± standard deviation (SD). Independent-samples t-test was used to compare two means. X2 test of significance was used in comparing proportions between two qualitative parameters. An independent sample parametric t-test was used in comparing data between the control and treatment groups. Confidence interval was set to 95%. P-value was considered significant as level of $p < 0.05$.

Results:

Analysis of tooth movement: It was found that tooth of the rabbits progressively moved to some limit, then (next to springs removal) tended to restore its premier place (relapse). In

our statistical analysis there was statistical significance in the comparison between the difference in the movements which occurred in the three groups (measured by18Q.CT and 38Q.CT) however there was no significant difference at the end of the 38th-day of movement (38d Q.CT) between the three groups (Table 1).

Analysis of bone density: It was observed that the Mesial root Bone density has a tendency to decrease with the progression of tooth movement, stabilizing in the control group with a lesser reductions in the bone density than other groups, after springs removal, during the period of relapse in the CKD groups with statistically significant difference between the three groups regarding bone density (Figure1).

As for the distal root Bone density, it showed a tendency to increase as the tooth movement progressed, stabilizing in the control group with values greater than the others, after removal of the springs, during the relapse period in the CKD groups (Figures 2), but a statistically significant difference was found between the two CKD groups regarding bone density bone where the bone density tended to increase as the tooth movement occurred, stabilizing in the CKD group with VD with values greater than the CKD without VD (Figure 2).

A significant correlation was found between OTM in relapse represented by the difference in final CT and bone mineral density in the three groups (Table 3).

Figure 4 shows the mean values of the number of osteoclasts, between the three groups (Control and CKD Groups). Significant differences were found in the number of osteoclasts when compared among days 1, and 38. Histological evaluation showed the difference in the number of osteoclasts and osteoblasts among three groups. (Fig. 3).

Table 1: Orthodontic tooth movement in mm:

	Control	CKD	CKD + Vit. D
After appliance removal after initial tooth movement: (18d Q.CT)			
Mean ± SD	**25.68 ± 1.12**	**21.82 ± 0.93**	**23.70 ± 1.45**
Range	**23.7-29.2**	**20.5-23.7**	**20.8-27.2**
P-value[1]		**0.000***	**0.002***
P-value[2]			**0.004***
Post relapse: (38d Q.CT)			
Mean ± SD	**26.33 ± 1.07**	**26.36 ± 1.14**	**26.22 ± 1.25**
Range	**24.5-28.5**	**24.2-28.4**	**24.1-28.5**
P-value[1]		**0.964**	**0.769**
P-value[2]			**0.752**
P-value[3]	**0.000***	**0.000***	**0.000***
Difference:			
Mean ± SD	**0.65 ± 0.05**	**4.54 ± 0.21**	**2.52 ± 0.20**
Range	**0.8-0.8**	**3.7-5.4**	**3.3-1.3**
P-value[1]		**0.000***	**0.000***
P-value[2]		**0.000***	**0.000***
P-value[3]			**0.000***

SD: standard diviation.

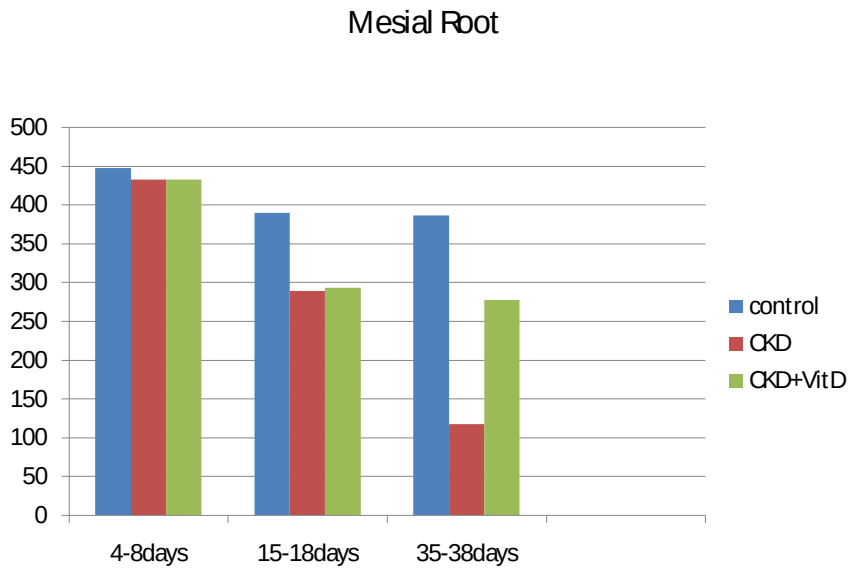

Figure 1: Mean of Bone Density in the mesial root of the lower first premolar:

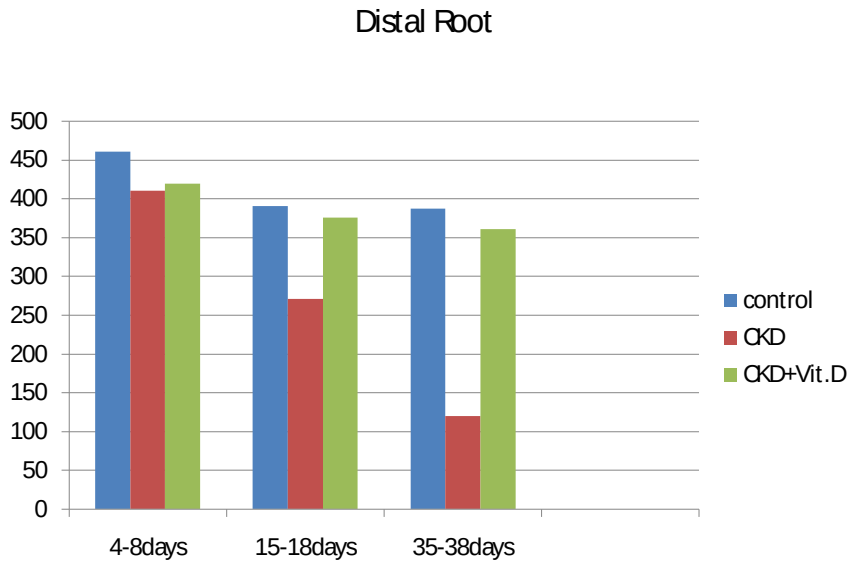

Figure 2: Mean of Bone Density in the distal root of the lower first premolar:

Table 2: Correlation between orthodontic tooth movement difference and bone density difference in all groups:

Groups	Orthodontic tooth movement difference	
bone density difference	r-value	P-value
Control	-0.822	0.003*
CKD	-0.657	0.032*
CKD + Vit. D	-0.726	0.015*

r-value: In statistics, the correlation coefficient r measures the strength and direction of a linear relationship between two variables on a scatterplot. The value of r is always between +1 and –1.

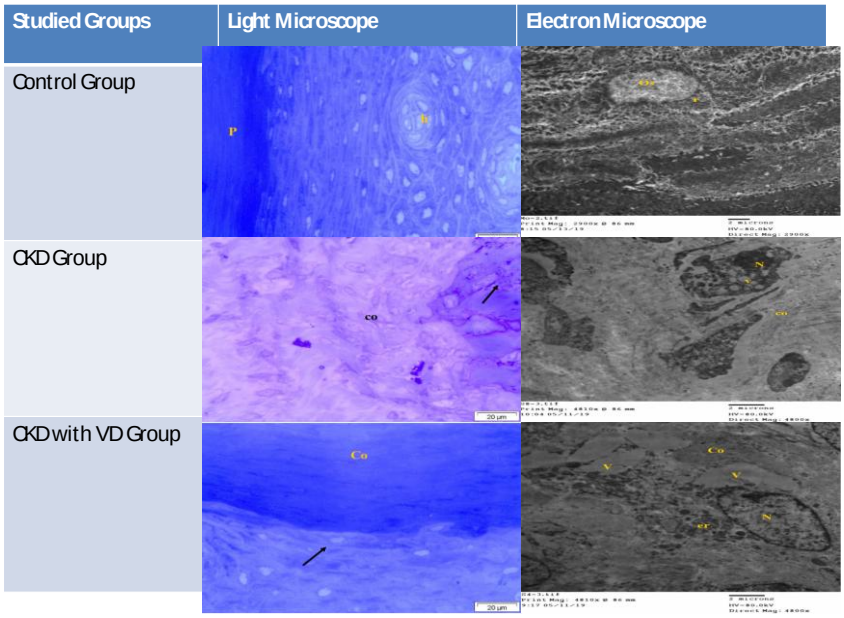

Fig (3): Histological analyses of bone specimens in the three groups by both light and electron microscope:

<u>In the control group:</u> Light micrograph of semi thin section of the tension side shows thickening of the periosteum (p) and the bony parts as a result of proliferation of the osteoblasts which differentiated from the fibroblasts of periosteum and have tendancy to form haversian canaliculi (h). (T.B. stain). Electron micrograph of the bony parts shows the osteoblasts (Os) rich with free ribosomes (r) and arranged in lamellar manner.

<u>CKD Group:</u> Light micrograph of semi thin section from the bony parts of the pressure side shows periodontal ligaments formed by fibrous collagenous tissue (co) and the attached bony part formed by degenerated osteoblasts and the ground matrix contain deeply stained granules (arrow). (T.B. stain). Electron micrograph from the bony parts of pressure side showed numerous osteoblasts aggregated to each other having miss shaped nucleus (N) and their cytoplasm having numerous small vacuoles (v) and RER (er). Notice the ground substance formed by light electron dense matrix (X).

<u>CKD with VD:</u> Light micrograph of semi thin section from the pressure side shows the periosteum formed by thick layer of collagen fibers (co) and the differentiated cells in the bony parts formed by large amount of matrix containing few osteoblast cells (arrow). (T.B. stain). Electron micrograph from the bony parts of the tension side shows the oestoblasts contain variable size vacuoles contain light electron dens material (v), RER (er) and dentate nucleus (N) and the ground substance contain collagen (co).

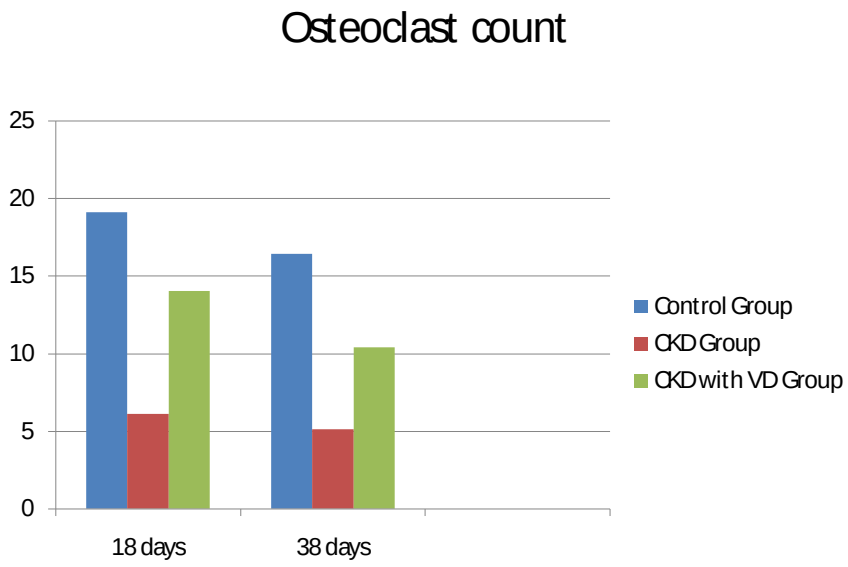

Figure 4: Osteoclast count means (cells/field) among three groups on day 18 and day 38:

Discussion:

In our study, the rationale of the uremic model was proven previous animal studies. [11]. Subsequently, this animal model was compatible to simulate metabolic disturbances in CKD patients.[12] This experiment emphasizes the assumption that local vitamin D injection could decrease the osteoclasts numbers during tooth movement in orthodontic relapse. Statistical analysis showed a significant difference between the control group and the CKD groups in both tooth movement and bone density. Uremia is known to be associated with decreased bone density. We found that uremia led to reduce bone regenerative capability and raise degenerative capability, suggesting that increased bone degenerative capability was formed when uremia was existed, while usage of locally injected Vit.D3 in those uremic models resulted in betterment in bone regenerative capability [6]. It has been reported that the vitamin D has health several functions as it has anti-inflammatory, antidiabetic, antioxidant, anticholesterol, anti-inflammatory, antiaging and antimicrobial properties. The results of this experiment showed a significant difference in the total number of osteoclasts between the three groups, with the mean osteoclast number being lower in the CKD group than in the control group and CKD with vitamin D in the previous mentioned dose. This might be due to the reduction of the osteoclastogenesis, that decrease osteoclast formation then differentiation; and so, the total number of osteoclasts was decreased [13]. Recently calcitriol was considered to be a promising choice in orthodontic treatments, and so we decide to evaluate the clinical utility of such local injected vitamin D in reducing relapse. The rationale about selecting the doses is referred to previous studies about VD, which showed its influence on bone density remodeling and effective within the normal physiological dose [6, 17]. For each of all groups, the OTM showed higher clinical net rate of movement in the CKD group versus in the Control and CKD with VD groups; however this movement was found to be lesser in the CKD with VD than in the CKD group. This finding is comparable to that produced by a calcitriol dose in cats that reported in previous study [14]. Our results regarding the effect of locally injected vitamin D and its inhibitory effect on osteoclastogenesis manifested by the significant reduction of osteoclasts in the CKD

with VD group in comparison to the CKD group is in agreement with other animal studies which have found that calcitriol induces bone resorption by stimulating the differentiation of osteoclasts [15]. Actually, the results of the present study confirm the findings of Khairallah et al. [6] and Pradeep et al [16], that found increase in bone deposition and decrease in bone resorption with improvement of bone mineral dinesity, with the use of low doses of vitamin D (calcitriol).

CONCLUSION: Currently, data about orthodontic treatment in uremia are limited. However as the advancement occurring in treatment of uremia, orthodontics treatment is becoming a necessary procedure, and therefore it is necessary to understand the bone abnormalities in those subjects. Our study can be concluded that non-invasive, loally injected Vitamin D will improve bone density by reducing osteoclastogenesis due to a decrease in the number of osteoclasts and therefore inhibiting relapse after orthodontic treatment in uremic rabbits.

Declaration:

Ethical regulations: This study design was approved by the Research Ethics Committee of the Faculty of Dentistry, Minia University, Minia, Egypt.

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