EVALUATION OF THE EFFECT OF CHOLECALCIFEROL ON TITANIUM IMPLANT OSSEOINTEGRATION (AN EXPERIMENTAL STUDY)

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ABSTRACT

Introduction: Cholecalciferol (Vitamin D3) is essential for bone mineralization and for the subsequent maintenance of bone quality. Supplementation with Cholecalciferol (vitamin D3) is reported to show positive effects on bone mineral density

Objectives: Evaluation of the effect of Cholecalciferol on dental implant osseointegration.

Materials and Methods: The study was conducted on fourteen adult male mongrel dogs.Dogs were divided equally into two groups, control group and study group, seven dogs in each group. Extraction of mandibular right premolar and insertion of immediate titanium implant had been done for all dogs in both groups, and then only the dogs of the study group received Cholecalciferol for four weeks. Histological and radiographical evaluations were carried out after twelve weeks postoperatively for both groups.

Results: Histological results revealed improved bone healing in the study group, as shown by marked osteoblastic activity and accelerated woven bone formation, and absence of chronic inflammatory cells. The highest rate of bone ingrowth occurred in the study group. Radiograghical evaluation revealed that the peri-implant bone density had increased significantly in the study group.

Conclusion: These results indicated that Cholecalciferol vitamin D3 has systemic effects on accelerating bone formation around titanium implant.

Keywords: Cholecalciferol, Vitamin D3, Dental implants, Osseointegration.

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INTRODUCTION

Over the last two decades, research has validated the success of osseointegrated implants as a viable replacement for partial and complete edentulism. Although techniques and materials have been developed which are capable of high degree of clinical success, the ultimate long-term success of implants is dependent upon the effort of both the patient and dentist in maintaining the health of peri-implant tissue (1). Recently, the clinical utilization of dental implants has accelerated and it is now a well recognized form of tooth placement, and a safe method for oral rehabilitation with high success rates (2).

Various factors may enhance or inhibit implant osseointegration. Factors enhancing osseointegration include implant-related factors such as implant design and chemical composition, topography of the implant surface, material, shape, length, diameter, implant surface treatment and coatings (3), the status of the host bone bed and its intrinsic healing potential (4), the mechanical stability and loading conditions applied on the implant (5), the use of adjuvant treatments such as bone grafting, osteogenic biological coatings and biophysical stimulation (6-8) and pharmacological agents such as simvastatin and bisphosphonates (9, 10). Supplementation with Cholecalciferol (vitamin D3) is reported to show positive effects on bone mineral density (11-15).

The term “vitamin D” actually refers to a group of fat-soluble vitamins. There are five different forms of vitamin D, but the two major forms are vitamin D2 (Ergocalciferol) and vitamin D3 (Cholecalciferol). Vitamin D2 is produced by plants, while vitamin D3 is produced by the skin of animals in response to sunlight (Ultra Violet light) exposure. UV light reacts with an enzyme called 7-dehydrocholesterol to create pre-vitamin D, which rearranges its structure to form vitamin D3, then converts vitamin D3 into a compound called calcitriol, which is the active form of vitamin D that is responsible for the numerous health benefits. After its conversion from Vitamin D3, calcitriol exerts its effects on the body by binding to and activating vitamin D receptors (VDRs), which are located in the nuclei of target cells (16).

The major physiological role of vitamin D is to facilitate the intestinal absorption of calcium, by stimulating the expression of proteins involved in calcium transport. Vitamin D also plays a crucial role in providing the proper balance of minerals necessary for bone growth and function. However, it turns out that VDRs are present in the cells of most organs in the body, suggesting that there is wide diversity in the types of responses that vitamin D3 can promote (17-19).

Vitamin D3 is recognized as a regulator of both osteoblast mediated bone formation and osteoclast mediated bone resorption (20-22) . Besides these, numerous other disease associations have been reported with VD3 deficiency, including cardiovascular disease, common obesity, and diabetes mellitus (23, 24). Therefore, the purpose of this study was to evaluate histologically and radiographically the role of Cholecalciferol on osseointegration on dental implants.

MATERIALS AND METHODS

This study was conducted on fourteen male mongrel dogs, about 18-24 months old, and average weight 10 to 12 kgs.

The animals were kept under the same nutritional and environmental conditions in the Animal house, Physiology Department, Faculty of Medicine, Alexandria University. The total number of dogs was divided equally into two groups each group consisted of seven dogs:

• Control Group: consisted of seven dogs which had extraction of mandibular right premolar and insertion of immediate titanium implant.

• Study Group: consisted of seven dogs which had extraction of mandibular right premolar, insertion of immediate titanium implant and received a daily dose of Cholecalciferol immediately after the operation.

Devarol-S ™ (Memphis Pharmaceutical & Chemical Industry, Cairo, Egypt) is a form of vitamin D, also called vitamin D3. Devarol-s ™ was provided in a form of 2 ml glass ampoule. Each ampoule contains Cholecalciferol (Vit.D3) 5mg (equivalent to200000 I.U.) in a sterile clear solution (Fig.1).

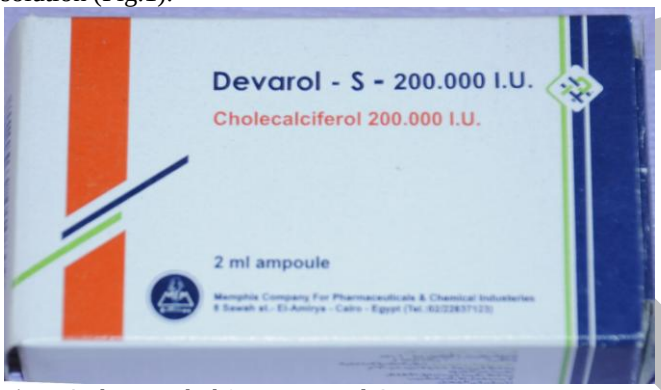

Fig 1: A photograph showing Devarol-S.

Dentis ™ (Dentis Co., Ltd. 951, Woram-Dong, Dalseo-Gu Daegu, South Korea) titanium implant is the implant of choice for this experimental study. This implant is designed with the following characteristics: Resorbable blast media(RBM) surface treatment whereby micro-particles of Hydroxyapetite-derived Beta-Tricalcium Phosphate are impregnated into the titanium surface through high pressure blasting techniques in order to obtain micro-surface roughness, safe cutting edge for reduction of bone stress, allowing smoother insertion, dome end to decrease perforation possibility,tapered body with optimized thread designs for easy initial fixation at the time of placement surgery and implant sizes range from 3.00-6.00 mm in diameter and from 8-14 mm in length.

Before surgery, all dogs were healthy as documented by a veterinarian report. The dogs were kept under the same nutritional and environmental conditions and were kept on the same balanced diet consisting of milk, broth and meat throughout the whole period of the study. Each animal received a dose of antibiotics in the form of ampicillin (25 mg/kg body weight; Epicocillin, provided by: Eipico Pharmaceutical Co., 10th of Ramadan city, Cairo Egypt) just before the operation by intramuscular injection. All operating procedures were performed under general anesthesia and sterile conditions in an animal theatre. Each animal was generally anaesthetized via intravenous injection of Thiopentone sodium (30 mg/kg body weight; Barbiturate provided by Glazer export Co. Dinshwa Waccha India).

After dogs were generally anesthetized, a blood sample about 10 ml was collected preoperatively from each dog in a sterile tube to measure serum calcium level. Atraumatic extraction of the mandibular right premolar was performed using forceps without any damage to adjacent soft or hard tissues and the extraction sockets were irrigated with normal saline. Drilling was performed as recommended by manufacturer and extended 3 mm beyond the root apex. Implants diameter and length ranged from 3.7 mm to 4.1 mm and from 10 mm to 12 mm respectively.

After the final drill, the implant was carried with its mount to the socket, and then pressed a little bit with threading movement clockwise until resistance was encountered. This was followed by removal of the implant cap and final seating of the implant by ratchet wrench till the implant shoulder is at the level of the alveolar bone crest and then screwing of the cover screw was performed followed by suturing of the wound. Study group received a daily dose of Cholecalciferol 12µg/kg/day by intramuscular injection immediately postoperatively (Fig.2).

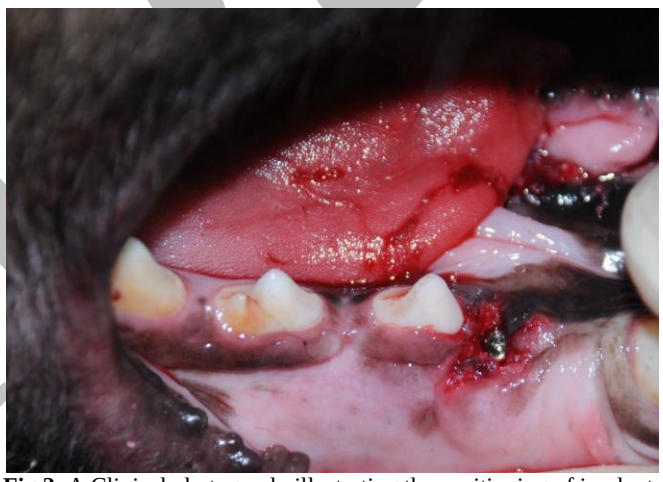

Fig 2: A Clinical photograph, illustrating the positioning of implant in the premolar area.

After the surgical procedure, each dog received the same course of antibiotics of ampicillin 25 mg/kg body weight for five days every eight hours. Ketolac (1 mg/kg body weight; Ketorolac Tromethamine by ELAMRIA Company) subcutaneous injection every twenty four hours was given as analgesic and anti-inflammatory drug to the animals for three days post-operatively. The animals of each group were isolated in separate cages to be kept under observation to assess the presence or absence of any post-operative complications as infection, wound dehiscence or implant rejection.

Then the animals were maintained on soft diet consisting of bread, milk and broth post-operatively for one

week, after that shifted to normal balanced diet again. Study group dogs received a day after day dose of Cholecalciferol 12 µg/kg/day by intramuscular injection for a period of 28 days. Blood sample about 10 ml was collected from each dog in a sterile tube to measure serum calcium level before sacrifice on the 12th week. The dogs were sacrificed by an over dose of Thiopentone sodium after twelve weeks.

Immediately after sacrifice, the implant-bearing areas were retrieved, labeled and immediately immersed in 10% neutral formalin to be radiographed with periapical x-ray films to evaluate bone density using the Image J software. (Fig.3 and Fig.4).

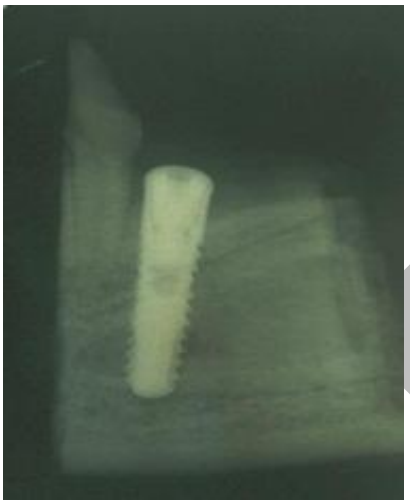

Fig 3: A periapical x-ray film of control group.

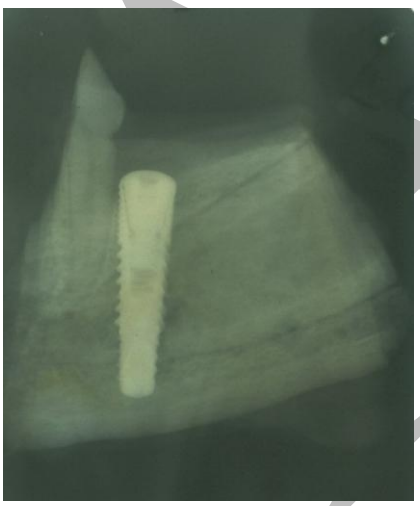

Fig 4: A periapical x-ray film of study group.

After the preparation of the specimens, tissue sections were cut at four microns thickness microscope then sections were stained by Hematoxylin and eosin stain (H&E) and Trichrome stain. Each section was examined under light microscope.

For statistical analysis, data were fed to the computer and analyzed using IBM SPSS software package version 20.0.Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and D'Agstino test. It reveals normal data distribution, and parametric tests were applied. For normally distributed data, comparison between two independent populations were done using independent t-test, also paired t-test is used to analyze two paired data. Significance of the obtained results was judged at the 5% level.

RESULTS

Clinical Results: All animals survived very well the experimental protocol of the present study and remained active and alert all over the course of experiment. The animals did not exhibit any clinical signs of infection, tissue dehiscence, discoloration or other tissue reactions surrounding the implants and this period went without complications in both groups.

Histological Results: After removal of the implant materials, the shape of the implant screw threads was distinctly visible on the walls of the bone block and pointed out the area of the implant osseointegrated interface. Bone growth and remodeling between the implant threads was already visible on the sample. After 3 months of healing, a complete bone volume was built between the implant threads and was observed as an imprint of the screw pattern of the implant.

In the control group a well marked implant threads in a direct contact with mature compact bone inside the concavities and convexities on both sides were found. Intact bone revealed that the bone in direct contact with the surface of the screw threads was without soft tissue intervening. (Fig.5)

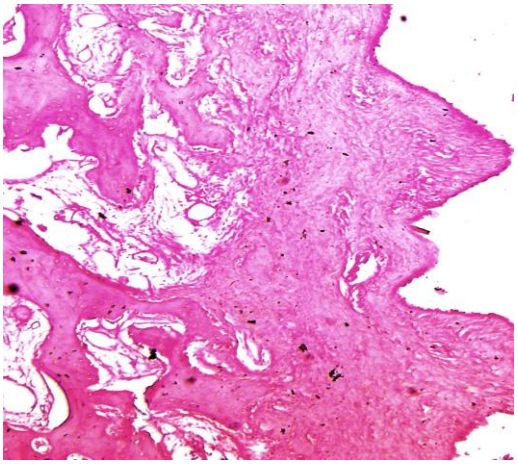

Fig 5: Photomicrograph of control group, showing organization and density of newly formed bone trabeculae with their ostocytes developing from the host bone(HB)and run towards the implant threads (arrows).(H&E X40)

In the study group microscopic examination revealed an intense invasion of numerous groups of well formed woven trabecular bone on both sides of the surface of the implant threads. A network of small marrow spaces containing fibrous tissue were scattered between trabecular woven bone. Profound dilated blood vessels were widely distributed among the newly formed trabeculae. An intense newly formed trabecular bone projects from the host. Projections were rich with blood supply. It is an evident feature of angiogenesis. The newly formed bone in the study group varies in size. It is well noted that the organized woven bone trabeculae were osseointegrated with host and directed towards the surface of the implant threads. The osteoid tissue is well detected with many osteocytes. Functioning osteoblasts bordered the new woven bone trabeculae. The early deposition of new calcified matrix on the implant surface is followed by arrangement of the woven bone trabeculae. (Fig.6)

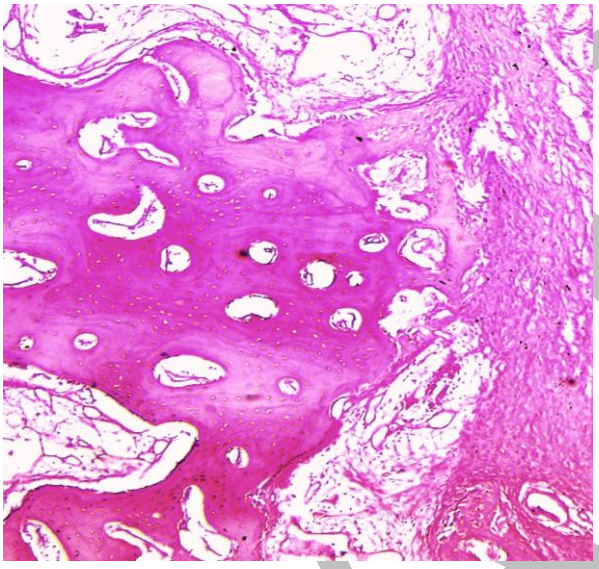

Fig 6: Photomicrograph of study group showing newer peri-implant bone (NB) and marrow spaces rich with vasculature in close vicinity of implant threads (arrows). (H&E X40)

Laboratory Results: There was no significant difference between the serum calcium levels in the control group and study group (Table 1).

Radiograghic Results: In comparison between the two groups, there was significant increasing difference in bone density in the study group (Table 2).

DISCUSSION

In the current work the overall histological observation in control group illustrated masses of newly formed bone with different degrees of maturity. The newly formed compact bone with Haversian system with volkman's canal and osteocytes with interstitial lamellae exhibited osseointegration in the bone of the socket. Implant osseointegration is the final goal of implant surgery and the prerequisite to achieve long-term success of endosseous dental implants.

Davis (25), Gailit (26) and Franchi M et al (27) reported that Peri-implant osteogenesis can be in distance and in contact from the host bone. Distance osteogenesis refers to the newly formed peri-implant bone trabeculae that develop from the host bone cavity towards the implant surface. In contrast, contact osteogenesis refers to the newly formed peri-implant bone that develops from the implant to the healing bone. The newly formed network of bone trabeculae ensures the biological fixation of the implant and surrounds marrow spaces containing many mesenchymal cells and wide blood vessels. A thin layer of calcified and osteoid tissue is deposited by osteoblasts directly on the implant surface. Blood vessels and mesenchymal cells fill the spaces where no calcified tissue is present.

Murai et al (28) were the first to report a 20-50 mm thin layer of flat osteoblast-like cells, calcified collagen fibrils and a slight mineralized area at a titanium implant-bone interface. The newly formed bone was laid down on the

	Serum calcium		
	Preoperative	**After 3 months**	**% of change**
Control (n = 7)			
Min. – Max.	10.90 – 11.20	10.90 – 11.20	-1.80 – 1.83
Mean ± SD.	11.03 ± 0.10	11.06 ± 0.10	0.27 ± 1.36
Median	11.0	11.10	0.0
p1	0.631		
Study (n = 7)			
Min. – Max.	10.90 – 11.20	11.0 – 11.20	-1.79 – 1.82
Mean ± SD.	11.04 ± 0.10	11.04 ± 0.08	0.01 ± 1.17
Median	11.0	11.0	0.0
p1	1.000		
t	0.277	0.302	0.384
p_2	0.786	0.768	0.707

Table (1): Comparison between the two studied groups according to serum calcium
t: Student t-test
p1: p value for Paired t-test for comparing between Preoperative and After 3 months
p2: p value for Student t-test for comparing between the two studied groups
*: Statistically significant at $p \leq 0.05$

	Control(n = 7)	**Study (n = 7)**	**t**	**p**
Bone density				
Min. – Max.	64.14 – 83.21	79.05 – 99.30		
Mean ± SD.	72.68 ± 6.44	89.36 ± 7.29	4.536*	0.001*
Median	71.44	87.65		

Table (2): Comparison between the two groups according to difference in bone density
t: Student t-test
*: Statistically significant at $p \leq 0.05$

reabsorbed surface of the old bone after osteoclastic activity. This suggested that the implant surface is positively recognizable from the osteogenic cells as a biomimetic scaffold which may favor early peri-implant osteogenesis. Osteoblasts cannot always migrate so rapidly to avoid being completely enveloped by the mineralizing front of calcifying matrix; these osteoblasts became clustered as osteocytes in bone lacunae.

The early deposition of new calcified matrix on the implant surface is followed by the arrangement of the woven bone and bone trabeculae. This is appropriate for the peri-implant bone healing process as it shows a very active wide surface area, contiguous with marrow spaces rich in vascular and mesenchymal cells. Marrow tissue containing a rich vasculature supports mononuclear precursors of osteoclasts so bone trabeculae remodel faster than cortical bone (27).

Initially, rapid woven bone formation occurs on implants to restore continuity, even though its mechanical competence is lower compared to lamellar bone based on the random orientation of its collagen fibers. Woven and trabecular bone fill the initial gap at the implant-bone interface. Arranged in a three-dimensional regular network, it offers a high resistance to early implant loading. Its physical architecture including arches and bridges offers a biological scaffold for cell attachment and bone deposition that is biological fixation. The early peri-implant trabecular bone formation ensures tissue anchorage that corresponds to biological fixation of the implant. This begins at 10 to 14 days after surgery (27, 29).

Vitamin D was originally described as a steroid hormone controlling calcium and phosphorus metabolism. Consequently, vitamin D deficiency is a pathogenetic factor for osteoporosis and the occurrence of fractures (30). However, preclinical studies suggest that vitamin D deficiency also negatively affects bone regeneration, including fracture healing (31) and the osseointegration of implants (32). While vitamin D supplementation is mandatory in pharmacologic osteoporosis therapy, few studies are available that would justify this treatment to support bone regeneration, and thus osseointegration (33).

Our histological findings in the study group showed intense and continuous new bone formation within all the threads of the implants. A well formed new woven bone with numerous osteoblasts and osteocytes were in close vicinity to implants threads.

A large number of osteoblast cells bordering the newly bone trabeculae as well as osteocytes within woven bone. These findings are consistent with Frenchi et al (27) and Probst (29). They revealed that osseointegrated implants is confirmed by the presence of medullary or marrow spaces containing osteoclasts, osteoblasts, mesenchymal cells and lymphatic/blood vessels next to the implant surface. During the remodeling of the peri-implant bone, new osteons circle around the implant with their long axes parallel to the implant surface and perpendicular to the long axis of the implants. Osteoid tissue is produced by osteoblasts suggesting that osteogenesis is underway. The remodeled bone can extend up to 1 mm from the implant surface.

In the present study, after three months, all the screws appeared osseointegrated, being almost completely covered by a compact, mature, newly formed bone. The peri-implant tissues showed areas of fibrous tissue alternating with direct bone contact. This was supported by Abrahamsson et al (34) and Vercaigne et al (35).

The histological observation in this study described peri-implant connective tissue corresponds to a marrow tissue in marrow spaces. The presence of a highly vascularised tissue rich in undifferentiated cells is helpful for the biological turnover of the peri-implant bone. Marrow spaces have always been observed around and next to the different implant surfaces; they were wide and filled by dense connective tissue or calcified tissue in 3-month-old samples. Their presence next to the implant surface during early peri-implant healing ensures a biological support for the turnover of mineralized tissues. During implant healing bone matrix mineralizes and envelops the osteoblasts, which produce the osteoid tissue and, if new bone is required, new osteogenic cells must migrate to that surface, and this was advocated by Davies in 2003 (36).

Interestingly microscopic examination in the study group of the current work revealed continuous newly formed bone is in direct contact with implant. It was originated from the compact bone of the socket bone. Complete osseointegration between bed bone and newly formed woven bone was originated from the bed bone with many marrow spaces within bony trabeculae. Vitamin D activates osteoblasts and increases the production of extracellular matrix proteins by osteoblasts. Vascularization is of critical importance for the process of osseointegration. Differentiation of osteogenic cells strictly depends on tissue vascularity.

Our findings were also in agreement with Franchi et al (27) who concluded that peri-implant bone contains regular osteons and host bone chips enveloped in mature bone. The bone-implant interface shows inter-trabecular marrow spaces delimited by titanium surface from one side and by newly formed bone from the other one rich in cells and blood vessels.

CONCLUSIONS

Within this context, it can be concluded that Cholecalciferol vitamin D3 has systemic effects on accelerating bone formation around titanium implant and can favor the biological turnover of the peri-implant bone.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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