EFFECT OF CALCITONIN INTRA MUSCULAR INJECTION ON BONE FORMATION AROUND TITANIUM DENTAL IMPLANTS IN DOGS (A HISTOLOGICAL STUDY)

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ABSTRACT

INTRODUCTION: Calcitonin is a major hormone secreted by thyroid gland responsible for bone formation and decrease of blood calcium level. It is a mixture of amino acids plus a modifying agent. It has been proved that the synthetic salmon calcitonin enhanced bone growth in depth as well as in width that is why they used it as a treatment for bone diseases and fractures.
OBJECTIVES: This study was done to evaluate histologically the effect of intra muscular (IM) calcitonin administration on bone healing around titanium dental implants.
MATERIALS AND METHODS: Twelve adult's Mongrel dogs received one implant in the maxilla in the edentulous space distal to the canine. Six dogs were randomly selected as the test group then they received a single daily dose of calcitonin 50 IU administered IM, for 28 successive days. And six dogs served as control group and no medications were injected. The animals were sacrificed 4, 8, and 12 weeks after surgery.
RESULTS: Through microscopical examination of specimens showed an apparent trend toward increased new bone formation in the osteotomy site in the calcitonin-treated group compared with the controls in fourth and eighth week intervals. While in twelfth interval there was a non-significant difference could be noticed between control and study groups concerning new bone formation which gave firm attachment between host bone tissue and the used implant.
CONCLUSIONS: This study demonstrated that using short, small dose of salmon calcitonin by daily intramuscular injection for 28 days enhanced bone mineral density when compared with the bone density without injection of this material.
KEY WORDS: Calcitonin, Dogs, Bone formation, Dental implants.

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INTRODUCTION

Edentation leads to impaired speech and masticatory ability and also may affect the psycho-social situation of the individual. Tooth loss can often be compensated by a conventional prosthesis; however, if this is not possible, a prosthesis supported by dental implants has been tried as an alternative (1).

Dental implant therapy is increasingly becoming main-stream care in today's society (2). In the last 3 decades, advances in biomaterial technology and continuous clinical research have provided clinicians with improved protocols to provide more advanced treatment options. Some of the original prerequisites of osseointegration have been reassessed to satisfy continuously increasing patient expectations of reduced treatment time, improved esthetics and increased comfort (3).

An endosteal implant is an alloplastic material surgically inserted into a residual bony ridge primarily as a prosthodontic foundation. The prefix endo means "within," and osteal means "bone". Many endosteal implant designs have been used in the past; including tapered pegs, pin shapes, and plate forms. Today, an endosteal implant in the shape of a tooth root is the design most often used in the restoration of partial or complete edentulous patients (4).

Osseointegration is the basic aim of placing implants in edentulous areas. It is defined as permanent incorporation of a non-biological component to carry unlimited functional load in endoprosthetic and exoprosthetic replacement in structure and function. It results in permanent attachment of titanium to human bone. In order to achieve proper osseointegration; certain criteria were reported such as proper diagnosis, treatment plan and follow up. It is so important to select the suitable cases for implant placement. Patients are preferred to be systemically free or at least well controlled. i.e. (not suffering from epilepsy or oral carcinomas or blood or bone diseases). Extreme ages are not preferred as very old or very young, below 18 years. Smoking should be prohibited as it interferes with healing (5).

The quantity and quality of the bone surrounding a dental implant influences implant osseointegration and affects the shape and contour of the overlying soft tissues and, consequently, the esthetic outcome. Only with careful considerations of the biologic principles of implant soft and hard tissues, as well as the appropriate selection of implant type and position, can a functional and esthetic treatment result is achieved (6).

An important feature of the osseointegration method is the emphasis put on efforts to accelerate bone formation. Recent studies are directed towards bone regeneration rather than bone replacement. Some authors (7) claimed that the topical application of biological growth factors may augment bone growth; while others stated that improvement of bone regeneration included the use of biologic mediators to improve the quantity and quality of regenerated bone (8).

In 1962 calcitonin was reported by Coop et al (9) to be a substance involved in calcium metabolism. As it acted as an antagonist to parathyroid hormone; calcitonin caused a decrease in serum calcium levels. Since its original discovery, calcitonin has been used to treat and prevent bone metabolism disorders, because it was believed to inhibit bone resorption (10), and because of its analgesic properties (11). The effects of calcitonin in healthy individuals have been observed to be the same as those observed in bone metabolism disorders (12). Other studies have reported that calcitonin also promotes new bone formation (13). Calcitonin, manufactured in the laboratory, is used in medicine to treat or prevent conditions that involve the loss of calcium from the bones. It has been used in the European Union (EU) for treating osteoporosis (a disease that makes bones fragile), Paget's disease (a bone disease that involves bone remodeling and can cause deformity), and hypercalcaemia (increased blood calcium) caused by cancer. It is also used to prevent acute bone loss due to sudden immobilization such as in patients with recent osteoporotic fracture. Calcitonin-containing medicines have been available in the EU as solutions for injection or infusion (drip into a vein) since 1973, and as a nasal spray since 1987. They are currently marketed in most EU countries (14).

McWhinnie (15) claimed that the alkaline phosphatase activity that corresponded to the calcification pattern might be influenced by calcitonin. Other authors (16) claimed that calcitonin acted by inhibiting osteoclastic bone resorption in ovariectomized animals. They related its effect to its influence on osteoclast formation and activity. Calcitonin was thought to cause disintegration of mature osteoclasts as well as inhibit the proliferation of progenitors and the differentiation of precursors to osteoclast cells. Other studies have reported that calcitonin also promoted new bone formation (13). Dogan et al (17) reported that calcitonin was capable of stimulating osteoblasts proliferation in experimental animals.

Two studies carried out on healthy animals, claimed that although salmon calcitonin had no influence on the initial period of bone healing around titanium implants inserted in the femur of healthy animals, it improved bone mass at the later stages of bone healing (18).

Thus, the aim of this study was to evaluate histologically the effect of calcitonin on bone healing around dental implants in dogs.

MATERIALS AND METHODS

The guidelines for the care and use of experimental animals according to the institution in which the work was done were followed.

Materials

Animals & experimental design:

- This experimental study was conducted on twelve healthy adult Mongrel dogs. The age of the dogs ranged from one to two years and with average body weight of 10 to12 kg.
- The dogs were examined by a veterinarian to exclude any diseased animal and they were kept under the same nutritional and environmental conditions in the experimental animal house at the Physiology Department, Faculty of Medicine, Alexandria University.

The dogs were randomly classified into two groups

I-The study group: Included six dogs that received one implant in the maxilla in the edentulous space distal to the canine. Then they received a single daily dose of calcitonin 50 IU administrated IM, for 28 successive days.

II-The control group: Included six dogs that received one implant in the maxilla in the edentulous space distal to the canine, and no medications was injected.

The Implant System

The implant used in this study was the Microdent implant system (Clearles Bahigas, Barcelona, Spain) GN implants, with a length of 9mm and a diameter of 3.9mm.

GN implants are two - piece titanium implants having hexagonal color coded Platform. The implant fixture is of the threaded-type with different thread depths for gradual fitting and stability. The implant surface is sandblasted except for the upper 2 mm portion which is electro-mechanically polished. The implants have sandblasted, large grit acid-etched surface and sterilized by gamma ray sterilization.

Calcitonin

Salmon calcitonin (Miacalcic, Novartis Pharma, Stein) is supplied as a sterile, colorless, clear, isotonic solution in a glass cartridge which is pre-assembled into 1 ml (equivalent to 50 IU) for IM injection. It is stored between 2° - 8°C.

Methods

Preoperative phase

The dogs were examined by a veterinarian to exclude any diseased animal. The animals were weighed to estimate the amount of anesthetic solution. The animals fasted the night before the surgical operation to prevent vomiting after anesthesia.

Operative phase

Anesthesia

The dogs received general anesthesia via intravenous (IV) injection of Thiopentone Sodium (Barbiturate provided by Glazer Exports Co. DinshawWaccha India).

Surgical procedure

1- The surgical procedure was performed under aseptic conditions for all dogs.
2- The oral mucosa was painted thoroughly with antiseptic Povidone Iodine (Betadine, the Nile Co. for Pharma, Cairo, Egypt. Under license from Mundi Pharma, AG, Basel, Switzerland) solution 10%, so as to protect the surgical field from microorganisms.
3- Drilling with the osteotomy drill size 3.80 mounted on low speed hand piece was performed in the edentulous space distal to the maxillary canine under cooling with sterile saline irrigant. A pumping motion was used while drilling to allow the osseous debris to be cleared from the surgical site.
4- The implant was inserted using an insertion torque not greater than 40 Ncm into the prepared socket with the vial cap and turned in a clockwise direction with slight apical pressure to gain stability of the implant till difficulty was encountered, and then the vial cap was removed.
5- For final seating of the implant in bone; the Ratchet driver combined with the hex driver was mounted onto the implant, and used till the implant body was flushed with the level of alveolar crest of bone.
6- The cover screw was then placed on top of the implant using the screw driver.

7- In the study group the dogs were injected IM in biceps femoris muscle with calcitonin 50 IU diluted in 0.9% saline and administrated in a single daily dose for 28 consecutive days.
8- In the control group the dogs were not injected with calcitonin, or any other solution.

Postoperative phase

1- Immediately after the surgical procedure, each dog received IM injection of ampicillin (Epicocillin provided by epico pharmaceutical Co, 10th of Ramadan City Cairo Egypt) (25mg/kg) for 5 days every 8 hours to prevent infection.
2- Diclofenac Potassium (NSAID, provided by Novartis, El Sawa St Amiria Cairo Egypt). 50mg IM injection was given every eight hours for five days to control postoperative pain.
3- The dogs were transferred to a clean cage to be kept under observation to assess the presence or absence of any signs of infection or wound dehiscence.
4- The dogs were fed with soft diet consisting of bread, milk and broth for the remaining period of the experiment.

Animal scarification

The dogs were sacrificed by over dose of the anesthetic solution in the following sequence: 4 dogs (2 from study group and 2 from control group) after 4 weeks, 4 dogs after 8 weeks, and finally 4 dogs after 12 weeks. The specimens were then prepared for histological examination.

Histological preparation phase

Twelve (6 control and 6 study) biopsied specimens of demineralized tissue sections were examined microscopically. The effects of calcitonin treatment were examined by using light microscope. H&E stain was used to evaluate granulation tissue formation after four weeks, new bone formation after eight weeks, and lamellar bone formation after twelve weeks.

RESULTS

I- Clinical Evaluation

All animals survived the experimental protocol in good health, and remained active and alert. In the immediate post-operative period, the animals were observed daily for the first week to evaluate the clinical signs of infection in or around the wound site. The post-operative period went uneventful without complications.

II- Histological Evaluation

Twelve (6 control and 6 study) biopsied specimens of demineralized tissue sections were examined microscopically. The effects of calcitonin treatment were examined by using light microscope. H&E stain was used to evaluate granulation tissue formation after four weeks, new bone formation after eight weeks, and lamellar bone formation after twelve weeks. Microscopical study of the slides showed an apparent trend toward increased new bone formation in the osteotomy site in the calcitonin-treated group compared with the control group.

Four weeks post-operative period

Group I (control group) (Fig. 1)

Microscopical examination of specimens revealed regular contact outline at the deepest area between the pre-existing implant and the regenerating tissue around it. Moreover, higher magnification showed the prominence of connective tissue fibers growing from the host tissue and directed to one of the implant grooves.

Figure (1): Light micrograph (LM) of control case after 4 weeks showing regular contact outline at the deepest area between the pre-existing implant and the regenerating tissue around it (H&E x100).

Group II (study group) (Fig. 2)

The examined specimens revealed well-formed connective tissue, with good orientation of the collagen fibers directed to the implant site. Beginning of osteogenesis process appeared by new bone formation inside the soft connective tissue. Higher magnification showed a well-formed piece of new bone trabeculae with osteoblast cell lining growing towards the implant site as well as osteocytes inside.

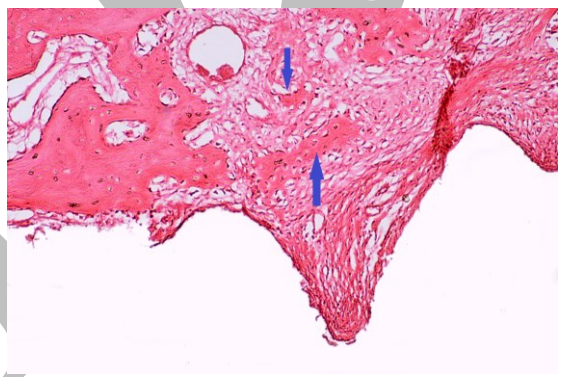

Figure (2): LM of study case after 4 weeks showing well-formed connective tissue projection, with good orientation of the fibers directed to the implant site. Beginning of osteogenesis process appeared by new bone formation (blue arrow) inside the soft connective tissue (H&E x100).

Eight weeks' post-operative period

Group I (control group) (Fig. 3)

The specimens revealed less organized bone formation around the pre-existing implant. Osteocytes appeared inside bone trabeculae. Higher magnification showed irregular bone trabeculae with wide marrow spaces.

Group II (study group) (Fig. 4)

The specimens revealed better organized new bone growing from the host side filling around the implant serrations. Higher magnification showed growing bone trabeculae lined with osteoblast cells and some osteocytes appeared inside.

Twelve weeks post-operative period:

Group I (control group) (Fig.5)

The specimens showed projections of bone remodeling around the site of implant serrations and alternating layers of new bone formation. Higher magnification showed resting lines inside well-formed bone trabeculae.

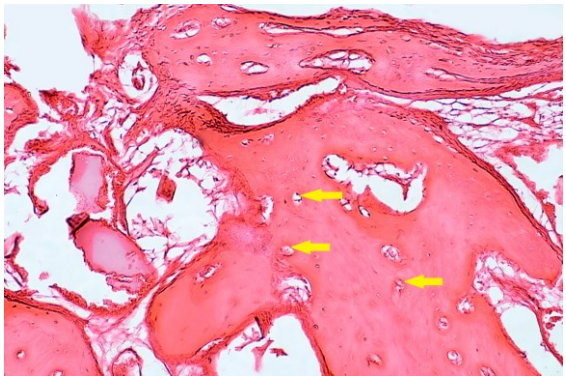

Figure (3): LM of control case after 8 weeks showing less organized bone formation around the pre-existing implant. Osteocytes (yellow arrow) appeared inside bone trabeculae (H&E x100).

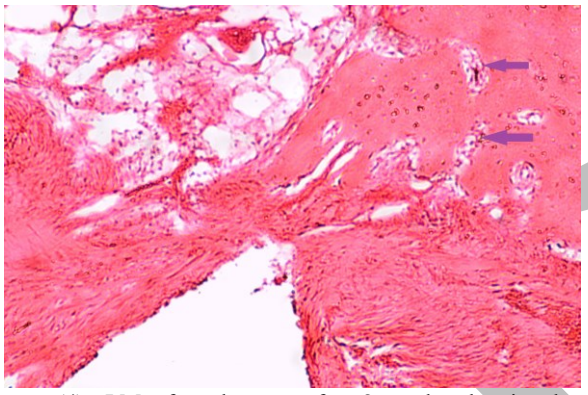

Figure (4): LM of study case after 8 weeks showing better organized bone growing from the host side filling around the implant serrations with cellular narrow marrow spaces (violet arrow) (H&E x100).

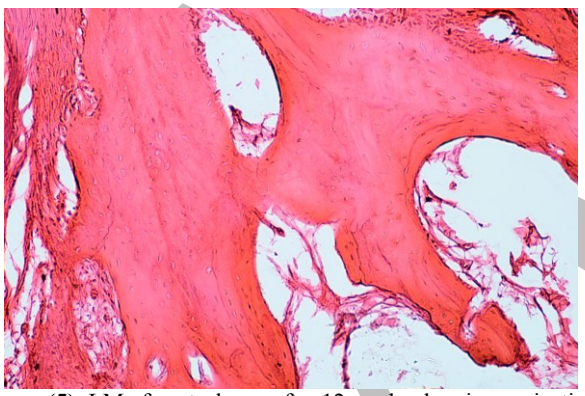

Figure (5): LM of control case after 12 weeks showing projections of bone remodeling around the site of implant serrations and alternating layers of new bone formation (H&E x100).

Group II (study group) (Fig.6)

The specimens showed projections of well-formed bony trabeculae directed to one of the implant grooves. Osteoblasts lining bone trabeculae appeared. Higher magnification showed some resting lines inside bone during osteogenesis process, which revealed successive layers of new bone formation.

By light microscopic examination after 12 weeks, no difference was noticed between control and study groups concerning new bone formation which gave firm attachment between host bone tissue and the used implant.

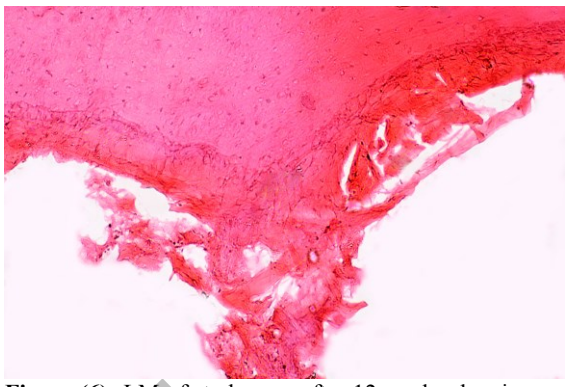

Figure (6): LM of study case after 12 weeks showing projection of well-formed bony trabeculae directed to the implant area (H&E x100).

DISCUSSION

The present study utilized the idea of benefits of Calcitonin hormone in bone formation in an attempt to enhance osseointegration around dental implant.

The choice of an animal model depends on several factors, while animal models may resemble themechanical and physiological human aspects, they provide only an approximation, with each animal having unique advantages and disadvantages, and the selection of the animal must take into account whether the focus is mechanical or biological (19).

The animal model of choice in this study was mongrel dogs; this choice was based on the availability of this species in Egypt as well as its frequent use in the regenerative experiments because the sequence of the healing process was proved to be similar in both humans and dogs. The only difference is that the healing is faster in dogs as the bone turnover being 2-2.5 times that of humans. This rapid healing in dogs was the reason for choosing such short follow up intervals (20).

In this study, a threaded-type implant was used as it allows percise placement, minimizes the shear stress, and provides better intial stability necessary for osseointegration at the implant bed (21).

The implant used was commercially pure grade IV titanium which is biocompatible due to its high corrosion resistance which was considered the most important property of titanium and its alloys. The ability of titanium to osseointegrate was attributed to the formation of oxide film which allowed fluids, proteins, hard and soft tissue to react or deposit on it (22).

Regarding the implant surface topography, acid-etched sandblasted implant surface was used since it has been emphasized by different authors that significant advantages exist for roughened titanium surfaced implants in comparison to smoother titanium implant surfaces. It has been reported that surface roughness widens the implant-bone surface and allows for the attachment, proliferation, and differentiation of osteoblasts; thus optimizing the osseointegration and enhancing the clinical function of the implant (23).

Furthermore, Cochran (24) in 1990 reported that sandblasted acid-etched surface implants tended to have slightly less crestal bone loss compared to other surface treatment.

In the current study implant length and diameter (3.9 mm in diameter and 9 mm in length) were the same for all dogs

in both groups, since the difference may influence pressure per unit area in the supporting bone (25).

Regarding the surgical procedure; it was done precisely and atraumaticaly at implant insertion, to achieve osseointegration. Shulman (26) in 1988 stated that trauma during surgery may lead to failure of the implant due to massive bone loss caused as a complication of that trauma.

Calcitonin is a natural hormone which is produced naturally by the thyroid gland in the body in order to increase blood calcium level. It inhibits osteoclastic bone resorption and enhances bone formation.Salmon calcitonin is the synthetic form of calcitonin used safely with minor side effects as it has been used to treat different bone diseases and as an analgesic to relieve bone pain , besides decreasing risk of bone fractures. Januario et al (18) in 2001 proved that the synthetic salmon calcitonin enhances bone growth in depth as well as in width in a previous study. That is why they used it as a treatment for bone diseases and fractures (27).

These advantages encouraged the idea of using salmon calcitonin in order to enhance bone formation around implants.

The injectable form of salmon calcitonin had been used in the present study via intramuscular route, as it is better absorbed rather than other routes to assure that the predetermined dose will reach the circulation, also to overcome local side effectsof other routes as decreased absorption and allergies. The concept of using I.M route was supported by Porgel and associates (28).

Salmon calcitonin is available as intranasal, intravenous, and intamuscular forms. The oral route is uneffective due to its decreased absorption, because it is broken down by aminopeptidases and proteases in the gastrointistinaltract. Although intranasal calcitonin is widely used and has wide patients acceptance, it is not as well absorbed as injectable calcitonin. The intranasal route seems to be more convenient and easier administration to majority of patients as it replaces multible injections. Intranasal administration may deposit a higher concentration of calcitonin near pain control centers in the brain where the drug may exert its primary analgesic effect, however it has got other side effects as it is not as well absorbed as injectable calcitonin, also causing rhinitis, nasal congestion, nasal dryness, sneezing and inflammation in nasal mucosa. That is why in the present study we preferred to inject dogs with salmon calcitonin via intramuscular route rather than using nasal spray (29).

Peichl et al (30) in 2005 reported that intermittent administration of 200 IU intranasal salmon calcitonin in postmenopausal women with osteoporosis reduced bone turnover serum markers, loss of further bone density, and pain.

The method of evaluation in this study was histolgical evaluation because it remained the most reliable method to determine the new bone formation steps (31).

Results obtained through microscopical examination of specimens showed an apparent trend toward increased new bone formation in the osteotomy site in the calcitonin-treated group compared with the controls. As in fourth week interval in the study group; the specimens showed beginning of osteogenesis process appeared by new bone formation.

Study group after eight weeks showed better organized new bone trabeculae. After twelve weeks; the study group showed successive layers of new bone formation.

By light microscopic examination after 12 weeks, there was a non-significant difference could be noticed between control and study groups concerning new bone formation which gave firm attachment between host bone tissue and the used implant.

Januario A.et al (18) in 2001 observed the effect of calcitonin on bone healing around titanium implants in an experimental study. He observed a growth in endosteal bone in width and height post operatively.

Previous studies were made by Fischer J.et al (32) in 1981 using salmon calcitonin to enhance bone formation around implants, the results showed enhancement in bone formation and maturation around implants placed. Similary in the current study dogs which were injected with salmon calcitonin and after twelve weeks, showed an enhancement in bone formation and maturation around implants post operatively.

Kaskani et al's (33) findings in 2005 revealed even a significant increase in bone mineral density (BMD) and a significant reduction in the specific alkaline phosphatase levels. Chesnut & coworkers (34) in 2005 added that calcitonin exhibited preservation of trabecular microarchitecture at the lower trochanter.

Comparable to its effect on osteoporotic females, calcitonin was able to produce significant increase in lumber BMD and reduce bone turnover in men with idiopathic osteoporosis. Moreover a significantly pronounced suppression of bone resorption was recorded (35,36). Experimentally, the effect of calcitonin on ovariectomized rat bone was restricted to the reduction of bone loss caused by estrogen deficiency (8).

There has been debate, whether the effects of calcitonin in healthy individuals were the same as those observed in bone metabolic disorders. Buclin et al (37) in 1987 and Glajchen et al (12) in 1990 reported that the effects of calcitonin in healthy individuals have been observed to be the same as those observed in bone metabolism disorders; whereas Avoli (38) in 1991 reported that calcitonin effects could be observed only in individuals with bone metabolism disorders.

In a study conducted by Dogan et al (17) in 2001, calcitonin proved to enhance osseous healing of the experimental cavities in the early stages, however there was no significant difference of osteogenic activity of calcitonin on the healing of osseous defects at the end of weeks 3 and 6. In contrast to the previous, calcitonin administration in distraction osteogenesis in rabbits revealed failure to enhance regenerate bone mineralization rate and tendency during bone lengthening.

Rgarding bone healing around titanium implants, it was found that salmon calcitonin had no influence on the intial period of bone healing around titanium implants inserted in the femur of healthy animals, still some improvement in bone mass might occur at the later stages of bone healing (18).

In accordance with Kaskani et al (33) in 2005, calcitonin had an effect on increasing bone mineral density. The timing of this improved density coincides with the proliferative and maturation phases of bone healing around dental implants, when local mesenchymal cells begin to

differentiate into fibroblasts and osteoblasts that secrete a collagenous matrix and contribute to its mineralization.

This finding is supported by Dogan et al (17) in 2001 who reported that calcitonin was capable of stimulating osteoblasts proliferation in experimental animals.

Trovas and associates (36) in 2002 showed that 200 IU of intranasal calcitonin daily for a period of 1 year produced increase in bone mineral density at the lumbar spine.

Januario A.and associates (18) have observed the effect of calcitonin on bone healing around titanium implants in an experimental study. The adult rabbits received titanium implants one in each femur. Bone analysis was done after 6,8,12 & 18 weeks. Growth in endosteal bone was observed in width and height after 12 & 18 weeks.

Based on the results of this study, it could be concluded that the administration of calcitonin systemically to healthy animals following insertion of titanium dental endosseous implants caused improvement of bone mass around the implant.

CONCLUSIONS

This study demonstrated that using short, small dose of salmon calcitonin by daily intramuscular injection for 28 days enhanced bone formation and maturation when compared with the bone without injection of this material.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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