EVALUATION OF EFFICACY OF ALENDRONATE VERSUS CALCITONIN ON IMPLANT OSSEOINTEGRATION IN OSTEOPOROTIC RABBITS

Maha Galal* and Abeer Kamal **

ABSTRACT

Background: Alendronate (ALO) is a second-generation bisphosphonate used widely in osteopenic individuals for increasing bone density. Calcitonin (CT) is approved for the treatment of osteoporosis and other diseases involving accelerated bone turnover. Successful implant osseointegration depend, in part, on state of host bone and its healing capacity, accordingly, it is necessary to clarify which of these two drugs is superior in management of osteoporotic peri-implant bone.

Objective: To compare the effects of Alendronate versus Calcitonin on implant osseointegration in osteoporotic bone.

Material and methods: Thirty female New Zealand White rabbits were used in this study. They were bilaterally ovarictomized (OVX) and fed on low Calcium diet (bran food) to establish osteoporosis model. Each animal received two implants in their tibia bilaterally. They were divided equally into 3 groups. Group I were considered as control (OVX) group. Group II received calcitonin (CT) I.M. and Group III received Alendronate (ALO) orally. At 12 weeks after implant insertion, the animals were scarificed. Ossiointegration among the three groups were evaluated through histomorphometrical analysis, scanning electron microscope (SEM), and dual-energy X-ray absorptiometry (DEXA) for measuring bone mineral density (BMD).

Results: Through histomorphometrical analysis, greatest mean area percentage of peri-implant bone was shown in (ALO) group, followed by (CT) group, the lowest effect was recorded in the control OVX group. Difference was statistically significant ($P<0.0001$). SEM results showed significant highest implant osseointegration value (Lowest gap distance) in (ALO) group, followed by (CT) group, then the control OVX group ($P<0.001$). BMD was decreased after OVX and then was increased after using each drug, this change was statistically significant. ALO group showed statistically significant higher mean BMD than (CT) group ($P<0.001$) with negative correlation between gap distance and BMD in both groups.

Conclusions: In osteoporotic conditions, both Alendronate and Calcitonin could effectively enhance osseointegration. Alendronate induced more pronounced effect than Calcitonin making it better choice for better implant osseointegration.


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INTRODUCTION

Osseointegrated implants represent an important alternative for the rehabilitation of partially or totally edentulous patients. Successful implant osseointegration mainly depends on the bone-to-implant contact and peri-implant bone mass and microarchitecture. (1) For implant stability, osseointegration is critical and is considered a prerequisite for implant loading and long-term clinical success of endosseous dental implants. (1, 2) Nevertheless, systemic alterations, such as osteoporosis, have been reported among factors potentially related to unsuccessful osseointegration. (2)

Osteoporosis is an osteometabolic disease that affects millions of people worldwide. It is prevalent in females and its incidence increases with age. (3) It is characterized by loss of bone mass, compromised bone strength, and anomaly in bony metabolism that adversely damage the osseointegration between prosthesis and host bone. This not only leads to failure of instant stability of the prosthesis but also high rate of loosening, shifting, and subsidence of the prosthesis in long-term results. (4)

After linear growth ceases, bone is in a constant state of remodeling, without overall loss of bone. This constant bone turnover is critical to overall bone health. Osteoporosis is essentially caused by aberrations in bone remodeling leading to bone fragility. (4, 5)

Rapid bone turnover can lead to an imbalance in bone renewal and to loss of connectivity within the trabeculae, that could irreversibly weaken the structural integrity of the bone. Such a weakening of bone microarchitecture has been found in early postmenopausal women. (5)

The diagnosis of osteoporosis was established based on the classical values of bone mineral density (BMD) achieved in the bone densitometry. Low bone mass is a major feature of osteoporosis. A strong inverse relationship exists between BMD and susceptibility to fracture. (6)

Treatment of osteoporotic patient depend not only on the result of the density, but also on factors that may be of high risk such as Estrogen deficiency, treatment with corticosteroid, hyperthyroidism and Hyperparathyroidism or of moderate risk as Physiological menopause, low calcium intake (<500-850 mg / day for prolonged periods), Smoking (> 20 cigarettes / day), Alcoholism and Osteopenic diseases. (6, 7)

As imbalanced bone turnover in osteoporosis plays an important role in implant failure, most therapeutic options are based on anti-catabolic or anabolic drugs, such as estrogen, calcitonin, bisphosphonates, and parathyroid hormone. (8-11) All these treatments have been demonstrated effective in improving implant osseointegration under osteoporosis conditions.

However, the anti-resorptive drugs, although potent in preserving bone mass, failed to promote new bone formation. Parathyroid hormone (PTH) is currently an approved anabolic agent for osteoporosis treatment, but the anabolic effect of PTH may reach its plateaus over time, and withdrawal of PTH was reported to cause deterioration of implant fixation in rats. (12)

Bisphosphonates represent the largest group of these anti-resorptive drugs currently in clinical use. (13) Bisphosphonates act by fixing to bone hydroxyapatite, inhibiting bone reabsorption by reducing osteoclast activity, facilitating their apoptosis and inhibiting their production from the corresponding hematopoietic precursor cells. They also reduce osteoblast apoptosis and stimulate the secretion of osteoclast recruitment inhibitors. Different bisphosphonates have significantly varying anti-resorption potencies and each individual drug has a variety of possible extra- and intracellular mechanisms. (14)

Alendronate (ALO) is a second-generation bisphosphate used widely in osteopenic individuals for increasing bone density. As a potent bisphosphonate, the ability of ALO to affect systemic
bone remodeling and inverting the negative effect of osteoporosis raises natural questions about the drug’s influence on dental implant osseointegration. Knowledge regarding the effect of systemic bisphosphonates, specifically ALO, on all 3 phases of osseointegration is still incomplete. Experimental studies have started to investigate peri-implant bone responses to alendronate-coated implants and its effect on peri-implant defect regeneration. These studies showed statistically significant increases in bone density and bone formation occurred with the alendronate-coated implants. Besides, unlike some other bisphosphonates, it was found that systemic alendronate does not interfere with mineralization or modeling in addition to anti-resorption activity. This may decrease the risk for failure of dental implant osseointegration.

The efficacy of salmon calcitonin (CT) has been confirmed by evidence-based clinical trials. CT could inhibit the bone resorption and the rate of bone turnover, resulting in relatively increase of bone formation by which enhancing the bone quantity and quality and consequently lowering the bone fracture rate. CT could not only increase bone quantity but also promote the osteointegration between host bone and prosthesis in different animal models including rats and rabbits.

However, other researches did not show positive efficacy of CT and ALO on peri-implant bone formation and osteointegration.

The increased frequency of both osteoporosis implant patients and administration of ALO and CT therapy within this patient group, requires a better understanding by the dental community, of how this disease, and specifically drug therapy, could affect osseointegration of dental implant and consequently its long-term stability under the circumstance of osteoporosis.

In view of this, the purpose of the present study was to compare the effects of Aendronate versus Calcitonin on implant osseointegration in osteoporotic bone.

**MATERIALS AND METHODS**

The study was performed according to the experimental research ethics committee of the Animal house, Faculty of Medicine, Cairo University.

Thirty pathogen-free adult New Zealand white female rabbits aged 9-12 months (2500-3500 gm), were used in this study. Each animal was weighed on arrival and checked by the veterinarian staff.

**Establishment of osteoporosis model**

Rabbits were randomly divided into 3 groups of 10 animals each, (groups I, II, and III), All animals underwent bilateral ovariectomies (OVX) operation and were also fed on low Calcium diet (bran food) for 45 days. Four weeks later, all rabbits underwent the Densitometric evaluation of bone mineral density (BMD) by dual-energy x-ray absorptiometry (DEXA) in tibia region (area of implant insertion) to confirm the existence of systemic bone mass loss. If the average BMD in the same group decreased by 20%, the osteoporosis model was confirmed to be established successfully.

Simultaneously, serum ionized Calcium level was detected on admission and after combined OVX with diet calcium restriction.

**Surgical procedure and drug administration**

Under General anesthesia, the animals were anesthetized using intramuscular injection of Ketamine (Ketamine HCL injection USP, Rotexmedica, Germany) and Xylazine (Rompun, Bayer AG, Leverkusen, Germany). Skin incision was made at the medial side of the knee joint under aseptic technique. After knee muscle and joint capsule dissection, the tibia platform was exposed clearly. From the center of the tibia platform, round bur was used to mark the implant site followed by
gradual drilling accompanied by copious irrigation with saline. Each animal received one Titanium implant in tibia bilaterally (3.4 mm in diameter and 8 mm in length) *. The implant was inserted and covered by covering screw. The same procedure was repeated in the other tibia fig. (1). After implantation surgery, rabbits in each group were administrated with drugs separately for 12 weeks as followings: Group I (OVX control group) subjected only to combined OVX and low calcium diet without any drugs. Group II, (CT), IM injection of Calcitonin (3.5 IU/kg)** in alternate days, for 12 weeks. Group III (ALO) received once weekly oral dose of 5 mg/kg of Alendronate sodium tablets,*** (5mg dissolved in 0.07 cc saline). The dose of the drugs was equivalent to human dose given that all rabbits weighed between 2-3 kg.**(25)

At 12 weeks after implant insertion, the animal were scarified and osseointegration was examined through histomorphometric and Scanning Electron microscope (SEM) analysis. Densitometric evaluation by DEXA was used in the tibia region for measuring BMD after drug treatment.

**Specimen preparation**

After animal scarification, the tibias were dissected and fixed in 10% formalin till examination. Samples were divided into two halves, half for Histomorphometrical analysis and the other, for SEM analysis.

**Histomorphometry**

Specimens were decalcified with 50% formic acid and 20% sodium citrate solution. After decalcification, implant was removed from bone tissue and bone was imbedded in paraffin. Serial sections were obtained and stained with H&E.**(26)**

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* Super Line Dentium Implant System, Dentium Co., Ltd. Seoul, Korea
** Miacalcic Ampoules, 50 IU synthetic salmon calcitonin. Novartis Pharma Stein. Egypt
***Osteonate drug tablets (Alendronate 91.35 mg as alendronate Sodium Trihydrate equal to Alendronic acid 70 mg) ADWIA Co. S.A.E 10th of Ramadan city.
The area percent of bone trabeculae was estimated using Leica Quin 500 analyzer computer system, (Leica Microsystems, Swizerland). The cursor was used to outline the areas of bone trabeculae, which were then masked by a blue binary color that could be measured by the computer. The image analyzer is calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. The area percent of bone trabeculae was estimated in 6 different fields in each slide using magnification (x200). Mean values and standard deviation were calculated for each group.

**Scanning Electron Microscope (SEM)**

The specimen containing implant was prepared according to the technique described by Hipp et al. Each specimen was dehydrated in a graded alcohol series for 10 hours and embedded in methyl methacrylate without decalcification. After polymerization sections were made through the longitudinal axis of the implants and through the surrounding non decalcified bone. The embedded tissue was cut into 150 μm thick section with low speed diamond wheel and then sanded on an abrasive paper to obtain a uniform surface finish. The spacemen then coated with a layer of gold with aid of magnetron spattering device and examined under high-resolution field emission scanning electron microscope (JEOL 6300F, Eching, Germany) connected to personal computer. The gap dimensions of implant to bone around all threads throughout the implant length were measured by μm using 4000-6000X and compared in the three groups. Spacemen that revealed complete osseointegration was further subjected to Energy-Dispersive X-ray spectroscopy (EDX), an analytical technique of SEM used for the elemental analysis or chemical characterization of samples. It was applied at different spectrums of bone -implant interface for confirmation of osseointegration.

**DEXA**

Using Dual Energy X ray Absorptiometry (DEXA) technique, bone mineral density was measured in tibia bone before OVX, after OVX, and around implants after using the drugs. Measured values were compared with each other during the same observation periods.

The data from each group were collected and statistically analyzed.

**Statistical analysis:**

Data of SEM, histomorophomery and DEXA were presented as mean and standard deviation (SD) values. One-way ANOVA test was used to compare between gap distances in the three groups. Repeated measures-ANOVA test was used to compare between Bone Mineral Density (BMD) values before OVX, after OVX, as well as after using the drugs. Tukey’s post-hoc test was used for pair-wise comparisons between the mean values when ANOVA test is significant. Pearson’s correlation coefficient was used to determine significant correlation between gap distance and BMD.

The significance level was set at P ≤ 0.05. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

**RESULTS**

**Histmorphometrical analysis**

Control OVX group (group I) revealed presence of residual thin bony structure without evident lamellar bone with non organized fibrous tissues.
Meanwhile CT group (group II), showed newly formed immature bone invading granulation tissues which recognized as a thin bone trabeculae and little lamellar structure with dilated blood vessels (fig:3) However, in ALO group (group III), there was ingrowth of more mature bone in direct contact with organized fibrous tissues. The bony trabeculae are more thick and containing increasing osteocytes in their lacunae with wide marrow spaces (fig:4).

The greatest mean area percentage of bone was recorded in ALO group (73.227±8.097), followed by CT group (63.582±6.665). The lowest value was recorded in the control OVX group (31.528±5.435). Analysis of variance (ANOVA) test revealed that the difference between the 3 groups was extremely statistically significant (p<0.0001). Moreover, Tukey’s post hoc test and unpaired Student’s t used for pair-wise comparison indicated a significant difference between the studied groups (Table 1, Fig. 5).

**TABLE (1)** Values of mean area percentage of bone trabeculae recorded in the different groups and statistical significance of the difference using ANOVA test

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Group CT</th>
<th>Group ALO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>31.528a</td>
<td>63.582b</td>
<td>73.227c</td>
</tr>
<tr>
<td>Std Dev</td>
<td>5.435</td>
<td>6.665</td>
<td>8.097</td>
</tr>
<tr>
<td>Std Error</td>
<td>2.431</td>
<td>2.981</td>
<td>3.621</td>
</tr>
<tr>
<td>Max</td>
<td>37.969</td>
<td>75.448</td>
<td>85.777</td>
</tr>
<tr>
<td>Min</td>
<td>24.692</td>
<td>55.876</td>
<td>61.792</td>
</tr>
<tr>
<td>2-s Range</td>
<td>21.74</td>
<td>26.659</td>
<td>32.388</td>
</tr>
<tr>
<td>F value</td>
<td></td>
<td>61.480</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>&lt;0.0001***</td>
<td></td>
</tr>
</tbody>
</table>

ANOVA test, *** extremely statistically significant
Tukey’s post hoc test: Means with different letters are significantly different.
TABLE (2) Pairwise comparison between the studied groups (Unpaired Student’s t test)

<table>
<thead>
<tr>
<th></th>
<th>t value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control OVX group versus CT group</td>
<td>9.1297</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>Control OVX group versus ALO group</td>
<td>10.4739</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>CT group versus ALO group</td>
<td>2.2528</td>
<td>0.048*</td>
</tr>
</tbody>
</table>

*statistically significant,

*** extremely statistically significant

SEM results

Gap distance at bone-implant contact (BIC) area

SEM results showed significant highest implant osseointegration value (Lowest gap distance) in (ALO) group, followed by (CT) group, then the control OVX group. (Fig 6)

At the apex, middle, cervical sites as well as mean of the three sites; control group showed the statistically significantly highest mean gap distance. CT Drug showed statistically significantly lower mean value. ALO Drug showed the statistically significantly lowest mean gap distance. (Table 3, fig 7)
After 12 weeks, successful osseointegration of the titanium implants was visualized in ALO group (III) at the ultrastructural level. Scanning electron microscopy showed well developed bone integrated at the implant surfaces and confirmed the intimate contact of the bone with titanium surfaces. EDX elemental analysis at area of complete osseointegration (no gap distance) revealed presences of bone mineralization elements concomitant with titanium element on the bone titanium contact area (BIC) ensuring maximum BIC in ALO group (Figure 8). On the other hand, EDX analysis of spectrum out of bone-implant interface revealed either mineralized bone elements or titanium elements (Figure 9A & B).

**TABLE (3) Descriptive statistics, results of ANOVA and Tukey’s tests for comparisons between gap distances (µm) in the three groups**

<table>
<thead>
<tr>
<th>Gap distance (µm)</th>
<th>Control</th>
<th>CT Drug</th>
<th>ALO Drug</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apex (Mean ± SD)</td>
<td>4.12 ± 0.51</td>
<td>0.88 ± 0.22</td>
<td>0.011 ± 0.006</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Middle (Mean ± SD)</td>
<td>5.97 ± 0.47</td>
<td>1.96 ± 0.52</td>
<td>0.60 ± 0.25</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Cervical (Mean ± SD)</td>
<td>8.73 ± 0.61</td>
<td>2.74 ± 0.64</td>
<td>1.33 ± 0.35</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean of the three sites (Mean ± SD)</td>
<td>6.27 ± 0.22</td>
<td>1.86 ± 0.21</td>
<td>0.65 ± 0.18</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*: Significant at P ≤ 0.05, Different letters in the same row are statistically significantly different.

![Fig. (7) Bar chart representing mean and standard deviation values of gap distance (µm) in the three groups](image1)

![Fig. (8) SEM – EDX Technique (Group III ALO): bone elements are integrated with titanium element denoting complete osseointegration at spectrum of BIC](image2)
Bone mineral density (BMD)

There was a statistically significant decrease in mean BMD after OVX in the two groups. Then a statistically significant increase in mean BMD was observed after using each drug. (Fig 10) The BMD after using both drugs showed non-statistically significant difference from BMD before OVX. Comparison between the two drugs revealed that ALO Drug showed statistically significantly higher mean BMD than CT Drug (Table 4 and Fig. 11).

Correlation between gap distance and (BMD) after using the drugs

There was a statistically significant negative (inverse) correlation between gap distance and bone mineral density. An increase in BMD is associated with a decrease in gap distance and vice versa. Table (5 and Fig12)

Fig. (9 A) SEM – EDX Technique (Group III ALO): bone elements only are detected at spectrum in bone area away from BIC

Fig. (9 B) SEM – EDX Technique (Group III ALO): titanium elements only are detected at spectrum in metal area away from BIC

Fig. (10) DEXA analysis of tibia bone (1) Treatment by Calcitonin (gp II) BMD 0.724 g/cm\(^2\) (2) Treatment by Alendronate (gp III) BMD 0.868 g/cm\(^2\)
Table (4) Descriptive statistics, results of repeated measures ANOVA and Tukey’s tests for comparisons between BMD (g/cm²) in each group before OVX, after OVX and after using the drug as well as comparison between the two drugs

<table>
<thead>
<tr>
<th></th>
<th>Before OVX</th>
<th>After OVX</th>
<th>After using Drug</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT Drug (Mean ± SD)</td>
<td>0.188 ± 0.048&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.084 ± 0.053&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.213 ± 0.031&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>ALO Drug (Mean ± SD)</td>
<td>0.222 ± 0.009&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.083 ± 0.044&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.254 ± 0.038&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>P-value (Comparison between the two drugs)</td>
<td>0.070</td>
<td>0.943</td>
<td>0.017*</td>
<td></td>
</tr>
</tbody>
</table>

*: Significant at P ≤ 0.05, Different letters in the same row are statistically significantly different

TABLE (5) Results of Pearson’s correlation coefficient for the correlation between gap distance (µm) and BMD (g/cm²)

<table>
<thead>
<tr>
<th>Correlation coefficient (r)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.456</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*: Significant at P ≤ 0.05

DISCUSSION

An improvement of osseointegration would be especially beneficial in compromised osseous situations such as osteoporosis. Studies analysing implant osseointegration in osteoporotic patients yielded disparity of results. Whereas some studies report increased implant loss rates for osteoporotic patients (2,4), others did not observe increased implant loss rates for these patients (29). However, it is assumed that the compromised bone metabolism in osteoporosis would negatively affect the healing process in the bone tissue surrounding the implants (2,4,5).

The aging population of humans has continually increased, and the number of osteoporotic patients is expected to increase considerably world wide. Consequently, there is increase in probability of deficient implant osseointegration sufferers.10 Thus, it has been imperative to develop potent therapeutic
options to improve implant osseointegration for osteoporotic patients who are frequently in active demand for dental implantation.

Many researches confirmed that both Alendronate sodium (ALO) and Salmon Calcitonin (CT) medicines could enhance the bone quality and quantity of osteoporotic bone at varying degree. Others tried to investigate the efficacy of different drugs used for management of osteoporosis on bone mass and implant osseointegration, among which, ALO and CT. On the account of previous experimental data, a crucial decision about the more effective drug is needed in order to maintain better implant stability in osteoporotic conditions. The present study compared the effects of Alendronate versus Calcitonin on implant osseointegration in osteoporotic bone.

In animals, osteoporosis may be experimentally induced by different methods. Ovariectomy (OVX) is the most widely used method to obtain a postmenopausal osteoporosis animal model. It is consistent, easy to induce, and highly reproducible. Moreover, significant bone loss can be obtained within a short period of time, and this variation remains stable after 3-4 months of OVX. In this sense, other authors have also employed combined methods to induce osteoporosis in several animal models as glucocorticoids in combination with OVX or diet calcium restriction in combination with OVX to achieve osteopenia to the degree seen in human osteoporosis. In the present research, combined OVX with low calcium diet was established as it furnishes an alternative method to develop a true osteoporosis without other potential detrimental effects of glucocorticoids. Besides, glucocorticoid treatment of rats would not induce osteopenia unless they receive a low-calcium diet.

Assessment of new treatments for osteoporosis, especially bone-forming agents, requires exploration of dynamic trabecular and cortical bone remodeling. Rabbits have often been used to study ingrowth of bone into implants and bone-implant interfaces. In contrast to rats, rabbits reach skeletal maturity shortly after complete sexual development, show substantial intracortical remodeling, and have a more rapid bone turnover than other rodents and even primates, hence were selected as experimental models in the current study.

Post ovariectomy estrogen privation period of 2-3 months, was established because following ovariectomy, bone resorption exceeds bone formation, increasing bone turnover and inducing rapid cancellous bone loss resulting in a severely rarefied trabecular network within three months. After osteoporosis model was established (BMD was decreased by 20% 4-6 weeks after ovariectomy and low calcium diet), Titanium implants were inserted into the proximal tibia metaphysis of all rabbits as a well characterized skeletal site for sex-hormone deprival induced bone loss. The animals were then divided to three groups, group I (control OVX), group II (CT group, IM) and group III (ALO, orally); the administrative dosage of the drugs were conversed from the equivalent dosage in human beings. At 3 months after implantation and drug therapy; duration that is enough for completion of the osteoconductive phase for implants in the osteoporotic model, all animals were sacrificed and specimens were harvested for examinations.

Implantation in the present work was unloaded. Implant loading leads to micro-motion at the bone-implant interface that if beyond the tolerable limit may significantly influences tissue differentiation around immediately loaded implants.

Success of osseointegration, a term coined by Branemark and co-workers in early 1960s, depends, among other factors, on the healing capacity of the bone tissue around the implant and represents a direct connection between the bone functional tissues and the biomaterial titanium. In the
current study, Osseointegration among the three groups were evaluated through histomorphometrical analysis, Scanning Electron Microscope (SEM) and Dual-energy X-ray absorptiometry (DEXA) for measuring bone mineral density (BMD).

Histomorphometry with its degree of objectivity, accuracy, reproducibility and suitability for statistical analyses was used for evaluation of osseointegration through assessment of percentage of peri-implant bone along implant surface. Results showed greatest mean area percentage of peri-implant bone in (ALO) group, followed by (CT) group, however, the lowest effect was recorded in the control OVX group. Difference was statistically significant. Owing to the fact that early peri-implant trabecular bone formation ensures tissue anchorage that corresponds to biological fixation of the implant, ingrowth of more mature bone found in (ALO) (group III), with thick bony trabeculae and increasing osteocytes in their lacunae, support the superior positive effect of ALO on osseointegration compared with CT (gpII).

These findings were supported by the results of SEM analysis. Evaluation of bone implant contact (BIC) or gap distance at bone-implant interface yielded significant highest implant osseointegration value (Lowest gap distance) in (ALO) group, followed by (CT) group, then the control OVX group. For further confirmation, Energy-dispersive X-ray spectroscopy (EDX) was used. Elemental analysis is an excellent way of assessing the elements and quality of new formed bone. In ALO group, through focusing on a spectrum of BIC area or bone –implant interface, EDX analysis revealed combination of Calcium and Phosphorous elements together with titanium elements. However, in areas out of bone-implant spectrum, EDX revealed either bone or titanium elements. On BIC evaluation, presence of Calcium and Phosphorous elements denote that mineralized bone had grown into implant surface, moreover, it could be an indicator for new formed bone quality that affect implant osseointegration and long term stability.

Studies involving elemental analysis used for this purpose are few. To our knowledge, no prior studies used this elemental technique for BIC evaluation in osteoporotic bone.

In support to our results, other studies, through histometrical and biomechanical analysis, demonstrated that animals treated with Alendronate presented greater resistance to implant removal, larger bone/implant contact area, larger bone area between the implant threads, and higher bone density in areas lateral to implant surface. Moreover, In a study conducted on osteoporotic rats using HA implants, ALO was found to be more effective than CT in osseointegration. Peri implant bone was evaluated only through histomorphometrical analysis with different drug dosage and different drug administration route in CT treated group.

On the other hand, other researches did not show significant positive efficacy of CT and ALO on peri-implant bone formation and osseointegration. Densitometric analysis of the OVX samples revealed OVX induced bone mass loss in examined regions. BMD was increased after using ALO and CT, this change was statistically significant. ALO group showed statistically significant higher mean BMD than (CT) group with positive correlation between osseointegration and BMD in both groups. These findings are consistent with those of a previous study, in which the induction of estrogen deficiency in ovariectomized rats caused bone structural alterations with lower density in long bones. Other studies, demonstrated increased bone mass density on using ALO due to its effect on decrease in bone remodeling, with consequent increase in the trabecular volume and the number of bone trabeculae.

Regarding the effect of CT on implant osseointegration, CT could inhibit the bone
resorption and the rate of bone turnover, resulting in relatively increase of bone formation, by which enhancing the bone quantity and quality in osteoporotic rats. (4)

In light of these findings, It seems reasonable to assume the superior effect of ALO over salmon CT on osseointegration of implants. This may attributed to the fact that ALO has a function of promoting bone formation process, while salmon CT plays no significant role in bone formation, and the role of ALO in inhibiting osteoclasts is much stronger than salmon CT. (16,17) Additionally, It was found that systemic ALO does not interfere with mineralization or modeling in addition to anti-resorption activity. This may decrease the risk for failure of dental implant osseointegration. Besides, ALO could normalize the high rate of bone turnover that characterizes osteoporosis. Consequently, enhancing the early stability of implants in patients with low bone mass. (15) Thus, ALO could reserve bone mass around the implant and help surrounding bone growth into the implants’ surface, thereby promoting efficient implant osseointegration. (24)

Although some cases that show association of the occurrence of osteonecrosis of the jaw and therapy with bisphosphonates have been reported, (40) it should be emphasized that these involved use of bisphosphonates with higher doses and in cases receiving intravenous bisphosphonates (pamidronate and zoledronic acid) for treatment of multiple myeloma and metastatic breast cancer or prostate cancer, while cases in patients receiving the drug orally for the treatment of osteoporosis are rare. (40)

It should be taken into account that, in the present study, the implants were inserted in the tibia bone. However, the accumulation of alendronate in the jaw bones may be greater than that observed in the long bones. In addition, the jawbones are more frequently handled and are more susceptible to infection.

This study has attempted to compare between the effects of two widely used systemically administrated anti-osteoporosis drugs on integration of host bone to implant surface in osteoporotic subject model. These was established through histomorphometrical and SEM analysis with SEM-EDX elemental technique for more confirmation of results at BIC area. In addition, monitoring BMD through DEXA was carried out during the same observation periods with correlation to gap distance or osseointegration. Our study showed valuable clinical findings allowing choice of the proper medication for patients with osteoporosis who have undergone prosthesis implantation.

CONCLUSION

Within the limitations of this study, it may be concluded that in osteoporotic conditions, both Alendronate and Calcitonin could effectively enhance osseointegration. Alendronate induced more pronounced effect than Calcitonin making it better choice for better implant osseointegration.

Recommendation further studies are required to demonstrate the long-term success of the implants and to assess osseointegration following implant loading in osteoporotic bone treated with both drugs. Besides, SEM studies of osseointegration, should include elemental analysis as it helps to assess the quality of formed bone at BIC, information that is very useful when it comes to applying research outcomes to everyday clinical practice.

REFERENCES


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