The Efficacy of Using Systemic Alendronate in Combination with Platelet-Rich Plasma in the Osteotomy Implant Site of Osteoporotic Rabbits

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Abstract: The success of osseointegration is mainly dependent on the state of the host bed. Therefore, concerns have been raised about osteoporosis, which is a condition believed to be associated with decreased bone quality and quantity. Alendronate (ALO) is a potent bisphosphonate that have the ability to control systemic bone remodeling and inverting the osteoporotic effect. Moreover, platelet-rich plasma (PRP) represents an autologous source of growth factors essential for bone regeneration. The purpose of this study was to investigate the effectiveness of using systemic Alendronate drug combined with topically applied platelet-rich plasma at the osteotomy implant site of osteoporotic rabbits. Thirty-two non-pregnant female New Zealand white rabbits, weighting 2500-4000 gm and aged 6-9 months, were selected. All rabbits undergo bilateral ovariectomies (OVX operation) and followed low Calcium diet for 6 weeks to induce osteoporosis. After incidence of osteoporosis the animals were randomly categorized into 4 equal groups : Group I; one implant placed in the animal right tibia without treatment (control group), Group II: one implant placed in the animal right tibia after topical application of PRP (PRP group), Group III: one implant placed in the animal right tibia followed by weekly single dose of 5 mg/kg oral Alendronate sodium dissolved in normal saline for 8 weeks (ALO group) and Group IV: one implant placed in the animal right tibia after topical application of PRP and using oral (ALO) dissolved in normal saline, 8 weeks after implantation (ALO+PRP group). Bone density at the bone-implant interface was evaluated at 2,4,6 &8 weeks after implantation by a radiographic analyzing software (Digora). All rabbits were sacrificed and Scanning Electron Microscopic (SEM) was used to evaluate implant-bone interface after 8 weeks of implantation. The greatest mean bone density was recorded in the ALO+PRP group, followed by the ALO group, then the PRP group, while the least value was the control group. the difference between groups was statistically significant (p<0.0001). The SEM results at 8th week after implantation showed the highest mean gap distance (μm) at the control group, followed by PRP and ALO groups whereas the ALO+PRP group showed the least distance. A statistically significant difference was seen between control group and all other groups while the difference between ALO group and ALO+PRP was insignificant. The combination of Alendronate and platelet-rich plasma could normalize the high rate of bone turnover that accompanied osteoporosis. Subsequently, enhancing the implants stability, reserving bone mass around the implant and activating bone growth on the implant surface, thereby promoting efficient implant osseointegration. [Amr elkarargy, Mostafa Omran. **The Efficacy of Using Alendronate in Combination with Platelet-Rich Plasma in the Osteotomy Implant Site of Osteoporotic Rabbits.** *J Am Sci* 2013;9(12):353-363]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 48

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1. Introduction

Bone healing is a complex process involving a number of cellular functions and mineralization followed by an eventual remodeling of the defect site to attain the original structure (Oda et al., 2009). Systemic disease such as diabetes mellitus and osteoporosis (OP) has been noted as potential conditions that delay bone healing. Moreover, OP-like conditions was often expressed in the geriatric female population as a common bone fracture (Nieves, 2005).

Recently, dental researches have been focused on OP as a disease characterized by reduction of bone mass, structure, and function. OP is thought to be a result of altered bone remodeling capacity, i.e., bone formation decrease while restorative capacity remains relatively constant (Wong et al., 2007).

OP was generally diagnosed by evaluation bone mineral density (BMD) accompanied bone densitometry. This correlation was assumed because of the strong inverse relationship exists between BMD and susceptibility to fracture. Although, OP affects both bone mineralization and architecture (Mellado-Valero et al., 2010).

Treatment of osteoporotic patient depends not only on bone density but also on other high risk factors affecting bone health. These high risk factors may include estrogen deficiency, corticosteroid therapy, hyperthyroidism and hyperparathyroidism. In addition, other moderate risk factors as physiological menopause, low calcium intake (<500-850 mg / day for prolonged periods), excessive smoking (> 20 cigarettes / day), alcoholism and osteopenic diseases

should be treated (Mellado-Valero et al., 2010 and Stein-Stein & Shane, 2003).

As imbalanced bone turnover in osteoporosis initiates implant failure, most therapeutic options are based on either anti-catabolic or anabolic drugs. Accordingly, estrogen (Qi et al., 2004), calcitonin (Duarte et al., 2003), bisphosphonates (Gao et al., 2009), and parathyroid hormone (Gabet et al., 2006) have been used effectively to improve implant osseointegration in osteoporotic conditions.

Currently, bisphosphonates represent the largest group of these anti-resorptive drugs used clinically (Rachner et al., 2011). Bisphosphonates act through fixing bone hydroxyapatite and inhibiting bone resorption by reducing osteoclastic cell activity. Moreover, they facilitate osteoclasts apoptosis and inhibit 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 (Mínguez-Serra et al., 2008).

Alendronate (ALO) is a second-generation bisphosphonate used widely in osteopenic patients to increase bone density. As a potent bisphosphonate, the ability of ALO to activate systemic bone remodeling and inverting the osteoporetic effect raises natural questions about the drug's influence on dental implant osseointegration. (Chacon et al., 2006) Studies have been conducted to investigate peri-implant bone responses to alendronate-coated implants and their effect on peri-implant defect regeneration. These studies showed statistically significant increases in bone density and bone formation surrounding alendronate-coated implants. (Meraw & Reeve, 1999 and Meraw et al., 1999)

Several studies demonstrated that the addition of specific growth factors might enhance regenerative process in both normal and osteoporotic bony defect (Jung et al., 2003). Growth factor is a naturally occurring substance, a protein, or a steroid hormone, capable of stimulating cellular growth, proliferation, and differentiation (Hauer et al., 2008).

Moreover, platelet-rich plasma (PRP) is a blood plasma that has been enriched with platelets. As a concentrated source of autologous platelets, PRP contains several different growth factors and other cytokines that stimulate healing of bone and soft tissue. Based on this principle, PRP are introduced to stimulate an extra-physiologic release of growth factors as well as optimize healing in chronic injuries. All of the known clinical applications of PRP highlight an accelerated tissue cicatrization due to the development of effective neovascularization, accelerated bone healing with fast tissue remodeling, and nearly total absence of infectious events (Choukroun et al., 2006).

The purpose of this study was to investigate the efficacy of using systemic ALO combined with topical PRP in the osteotomy implant site of osteoporotic rabbits.

2. Material and Methods
Induction of osteoporotic-like condition

Thirty-two non-pregnant female New Zealand white rabbits, weighting 2500-4000 gm and aged 6-9 months, were chosen as studied subjects. The treatment of the animals was approved by the Ethics Committee of the Animal House, Faculty of Medicine, Cairo University.

All rabbits undergo bilateral ovariectomies (OVX operation) (Mosekilde et al., 1993) and received 2 daily doses of 50 mg/kg ceftriaxone for 4 days as prophylaxis. The animals followed low Calcium diet (bran food) for 6 weeks to induce osteoporosis (Robert et al., 2005).

Densitometric evaluation of bone mineral density (BMD) by dual-energy x-ray absorptiometry (DEXA) (Hologic, Waltham, MA, USA) were performed at tibia of all rabbits (area of implant insertion). This method used to confirm the existence of systemic bone mass loss where BMD should be decreased by 20% to conform to osteoporosis (Jee & Yao, 2001).

Study groups

After Induction of osteoporosis the animals were randomly categorized into 4 equal groups:

Group I: included eight rabbits received one implant in right tibia (control group).

Group II: included eight rabbits received one implant in right tibia after treating osteotomy with PRP (PRP group).

Group III: included eight rabbits received one implant in right tibia followed by weekly single dose of 5 mg/kg oral Alendronate sodium dissolved in normal saline (Osteonate, Adwia Co. Egypt) for 8 weeks. The dose was calculated according to study of Paget and Barnas (1964), where a 1.5 kg body weight rabbit received 0.07% of the dose required for of 70 kg body weight human.

Group IV: included eight rabbits received one implant in right tibia after treating osteotomy by PRP and followed by single weekly dose of 5 mg/kg oral Alendronate sodium dissolved in normal saline for 8 weeks.

Preparation of PRP

PRP was prepared from rabbit's own blood for groups II &IV. Thus, venous blood (from the central ear vein) was drawn out in sterile containers (S-Monovette-Sarstedt, Germany) containing 1 ml anticoagulant citrate dextrose (CPDA-1).The PRP was

separated by centrifuge at aseptic conditions. The first spin was at 2400 rpm for 10 minutes, where the erythrocytes were separated from platelet poor plasma. At the second spin (3600 rpm for 15 minutes), the PRP was separated from platelet poor plasma. Finally, the PRP was activated by 10% $CaCl_2$ solution.

The implants were immersed in the activated PRP solution, avoiding any contact with the container walls. In addition, PRP was slowly injected at low pressure into the osteotomy immediately before implant placement (Khalil, 2013).

Surgical protocol

Under aseptic conditions the surgical procedure was carried out under general anaesthesia produced by an intramuscular injection of Xylazine (Chanazine, Chanelle Pharmacuetical, Ireland) 5mg/kg body weight and ketamine hydrochloride (Ketamine, Pharmazeutische Pröparate, Germany).30mg/kg body weight. Local anesthesia with 1ml of 5% Xylocaine (Astra, Sweden) was administrated to the tibial metaphysis where the implants were inserted.

Once general anaesthesia was established, the medial aspects in the region of the proximal tibia were shaved; the skin was carefully swabbed with mixture of iodine and 70% ethanol. A 30 mm incision along the medial aspect of the proximal tibia was extended and advanced down to the periosteum. A subperiosteal dissection was then advanced up to the inferior attachment of the knee joint capsule and laterally to the full extent of the flat medial bone surface.

Thirty-two titanium implants of 4.2 mm diameter and 8.0 mm length (Implantium, Dentium, Seoul, Korea) were inserted under copious irrigation with sterile saline according to the manufacturer's instructions. The prophylactic administration of procaine penicillin (Wyeth Pharmaceuticals, Parramatta, New South Wales.), 60 000 units/kg intramuscularly, preceded the surgery and continued for three postoperative days to reduce the potential for wound infection.

Radiographic Examination

It was performed to examine the density at implant-bone interface after 2,4,6&8 weeks of implantation.

Each rabbit was radiographed by X-RAY machine (Orix, Italy), image plate (sensor) with exposure time 0.5 sec at 70 Kv & 8 mA. The x-ray distance was standardized by placing the long-cone vertically at 90 degrees to a flat surface where the tibial bone was placed above the plate.

A radiographic analysis software (Digora v. 2.8, Soredex, Finland) was used for relative density measurements of bone-implant interface. An imaginary density area was drawn at the implant-tissue interface, extending from the coronal end to

apical end of the implant as close as possible to the implant.

A histogram appeared on one side of the image indicating the mean gray-scale values (0-255) within bone-implant interface. Different readings were taken from both sides of the implant and its apical part, and the mean of the readings were further used.

Animal sacrifice

All rabbits were sacrificed at the end of 8th week using an intramuscular injection of overdose of 60mg/ml/kg body weight sodium phenobarbitone (Phenobarbitone, Fawns & McAllan Pty Ltd, Melbourne, Victoria).

Scanning Electron Microscope (SEM)

The specimen containing the implant was prepared according to the technique described by Hipp et al. (1987). Each specimen was dehydrated in a graded alcohol series for 10 hours and embedded in methyl methacrylate without decalcification. After polymerization, the specimens were sectioned through the longitudinal axis of the implants and their 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. Further, the specimen was coated with a layer of gold using magnetron spattering device. Finally, the specimen was examined under high-resolution field emission scanning electron microscope (SEM, JXA-840A, JEOL, Japan), connected to a personal computer. The mean gap distance (μm) between the bone and implant in areas among the five threads was calculated using 4000-6000X and compared in all groups.

Statistical Analysis

Analysis of data was performed using SPSS 17 (Statistical Package for Scientific Studies) for Windows.

Description of variables was presented as follows:

- Descriptive analysis was in the form of mean and standard deviation (SD). Data were explored for normality of variance using Kolmogorov-Smirnov test of normality. The results of Kolmogorov-Smirnov test indicated that most of data were normally distributed (parametric data) so parametric tests were used for the comparisons.

- Comparison between quantitative variables was carried out by analysis of variance (ANOVA) test for independent samples. Tukey's post hoc test was performed when ANOVA test yielded significant results.

3. Results

A-Bone density (Mean grey scale value) at different time intervals

The greatest mean bone density was recorded in the ALO+PRP group, followed by the ALO group,

then the PRP group and finally the control group was the least. In all groups, bone density tended to increase by time with the least value recorded at 2 weeks and the maximum values obtained after 8 weeks (Table 1, Fig.1)

Table (1) Mean and standard deviation of grey scale in all groups in different intervals

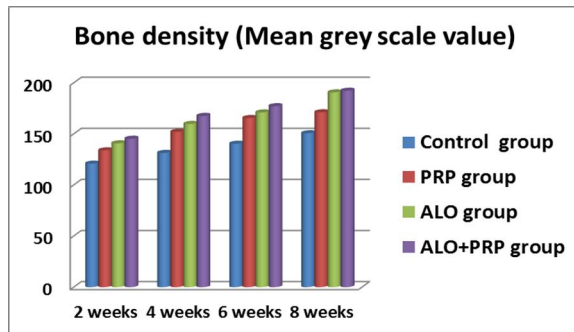|	Control group	PRP group	ALO group	ALO+PRP group
2 weeks	120.875 ±4.64	133.875 ±4.94	140.75 ±6.39	145.25 ±4.86
4 weeks	131.375 ±3.46	152 ±5.55	159.75 ±5.5	167.625 ±5.18
6 weeks	140.375 ±6	165.5 ±4.17	171 ±5.88	177.125 ±5.38
8 weeks	150.625 ±3.42	171.125 ±5.14	190.625 ±4.37	192.25 ±5.26

Fig. (1) Mean grey scale in all groups in different intervals

I- Comparison in mean grey scale of all groups at 2 weeks

The greatest mean bone density was recorded in the ALO+PRP group, followed by the ALO group, then the PRP group, with the least value obtained in the control group. Analysis of variance (ANOVA) test revealed that the difference between groups was statistically significant (p<0.0001). Tukey's post hoc test revealed that the difference between means of PRP group and ALO group was not significant and that the difference between ALO group and ALO+PRP was also insignificant (Table 2, Fig.2)

Table (2) Grey scale in all groups at 2 weeks and statistical significance of the difference using ANOVA test

2 weeks	Control group	PRP group	ALO group	ALO+PRP group
Mean	120.88[a]	133.88[b]	140.75[b,c]	145.25[c]
SD	4.64	4.94	6.39	4.86
Min	115.00	128.00	133.00	139.00
Max	131.00	141.00	151.00	152.00
F value	32.73			
P value	<0.0001***			

*** Statistically high significant

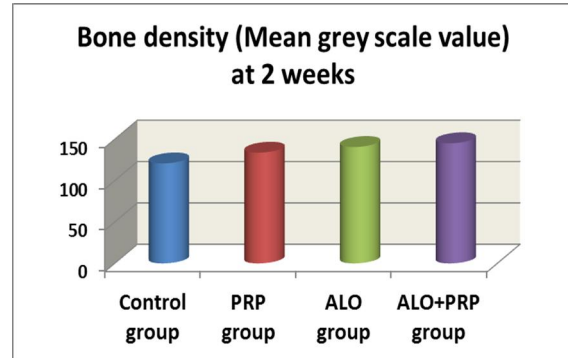-Tukey's post hoc test: means with different letters are significantly different.

Fig. (2) Mean grey scale in all groups at 2 weeks

II- Comparison in mean grey scale of all groups at 4 weeks

The greatest mean bone density was recorded in the ALO+PRP group, followed by the ALO group, then the PRP group, with the least value obtained in the control group. Analysis of variance (ANOVA) test revealed that the difference between groups was statistically significant (p<0.0001). Tukey's post hoc test revealed that the difference between each two groups was statistically significant (Table 3, Fig.3)

Table (3) Grey scale in all groups at 4 weeks and statistical significance of the difference using ANOVA test

4 weeks	C group	PRP group	ALO group	ALO+PRP group
Mean	131.38a	152.00b	159.75c	167.63d
SD	3.46	5.55	5.50	5.18
Min	127.00	147.00	152.00	161.00
Max	136.00	162.00	169.00	177.00
F value	77.7			
P value	<0.0001***			

*** Statistically high significant

-Tukey's post hoc test: means with different letters are significantly different.
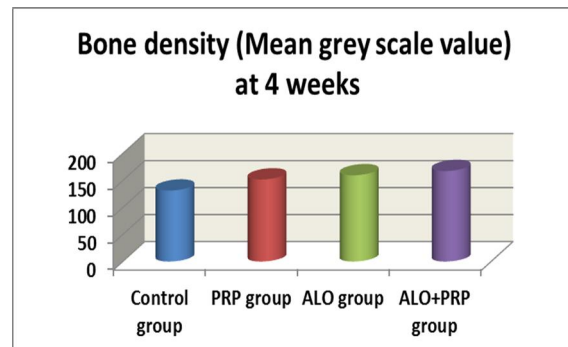

Fig. (3) Mean grey scale in all groups at 4 weeks

III- Comparison in mean grey scale of all groups at 6 weeks

The greatest mean bone density was recorded in the ALO+PRP group, followed by the ALO group, then the PRP group, with the least value obtained in the control group. Analysis of variance (ANOVA) test revealed that the difference between groups was statistically significant ($p<0.0001$). Tukey's post hoc test revealed that the difference between means of PRP group and ALO group was not significant and that the difference between ALO group and ALO+PRP was also insignificant (Table 4, Fig.4)

Table (4) Grey scale in all groups at 8 weeks and statistical significance of the difference using ANOVA test

6 weeks	C group	PRP group	ALO group	ALO+PRP group
Mean	140.38[a]	165.50[b]	171.00[b,c]	177.13[c]
SD	6.00	4.17	5.88	5.38
Min	132.00	161.00	164.00	169.00
Max	149.00	171.00	179.00	184.00
F value	71.19			
P value	p<0.0001***			

*** Statistically high significant

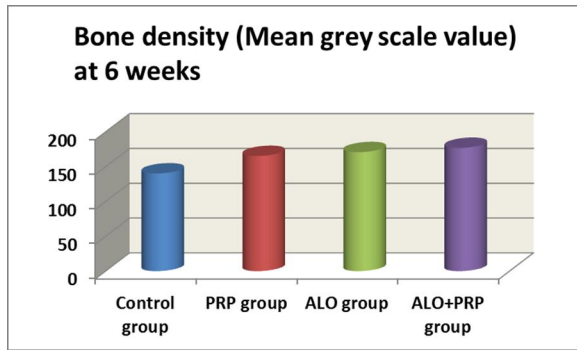-Tukey's post hoc test: means with different letters are significantly different

Fig. (4) Mean grey scale in all groups at 6 weeks

IV-Comparison in mean grey scale of all groups at 8 weeks

The greatest mean bone density was recorded in the ALO+PRP group, followed by the ALO group, then the PRP group, with the least value obtained in the control group. Analysis of variance (ANOVA) test revealed that the difference between groups was statistically significant ($p<0.0001$). Tukey's post hoc test revealed that the difference between ALO group and ALO+PRP was insignificant (Table 5, Fig. 5).

Table (5) Grey scale in all groups at 8 weeks and statistical significance of the difference using ANOVA test

8 weeks	C group	PRP group	ALO group	ALO+PRP group
Mean	150.63[a]	171.13[b]	190.63[c]	192.25[c]
SD	3.42	5.14	4.37	5.26
Min	147.00	166.00	186.00	187.00
Max	158.00	179.00	199.00	201.00
F value	143.96			
P value	<0.0001***			

*** Statistically high significant

-Tukey's post hoc test: means with different letters are significantly different.
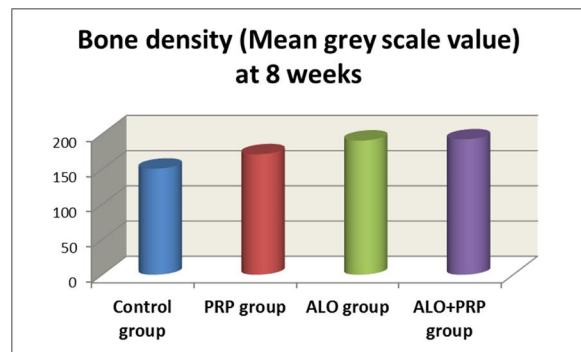

Fig. (5) Mean grey scale in all groups at 8 weeks

B-Change by time in mean bone density
i- Control group

Bone density tended to increase by time with the least value recorded at 2 weeks and the maximum values obtained after 8 weeks. Analysis of variance (ANOVA) test revealed that the difference between groups was statistically significant ($p<0.0001$). Tukey's post hoc test revealed that the difference between each two intervals is statistically significant (Table 6, Fig.6).

Table (6) Grey scale in control group throughout the experiment and statistical significance of the difference using ANOVA test

Control	2 weeks	4 weeks	6 weeks	8 weeks
Mean	120.88[a]	131.38[b]	140.38[c]	150.63[d]
SD	4.64	3.46	6.00	3.42
Min	115.00	127.00	132.00	147.00
Max	131.00	136.00	149.00	158.00
F value	63.44			
P value	<0.0001***			

*** Statistically high significant

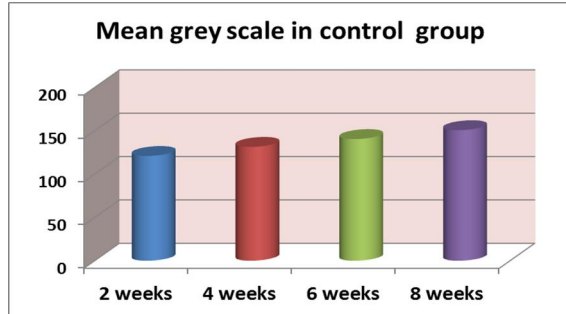-Tukey's post hoc test: means with different letters are significantly different

Fig (6) Mean grey scale in control group throughout the experiment

ii- PRP group

Bone density tended to increase by time with the least value recorded at 2 weeks and the maximum values obtained after 8 weeks. Analysis of variance (ANOVA) test revealed that the difference between groups was statistically significant (p<0.0001). Tukey's post hoc test revealed that the difference between each two intervals is statistically significant, except for the difference between 6 and 8 weeks (Table 7, Fig.7).

Table (7) Grey scale in PRP group throughout the experiment and statistical significance of the difference using ANOVA test

PRP	2 weeks	4 weeks	6 weeks	8 weeks
Mean	133.88^a	152.00^b	165.50^c	171.13^c
SD	4.94	5.55	4.17	5.14
Min	128.00	147.00	161.00	166.00
Max	141.00	162.00	171.00	179.00
F value	88.68			
P value	<0.0001***			

*** Statistically high significant

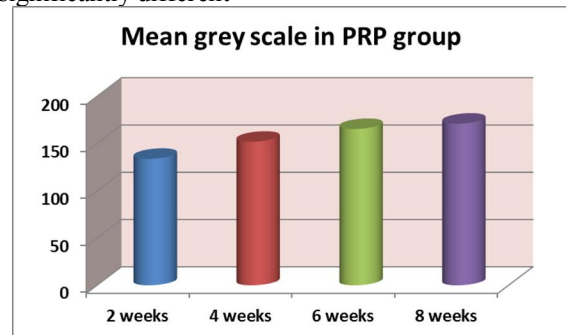-Tukey's post hoc test: means with different letters are significantly different

Fig. (7) Mean grey scale in PRP group throughout the experiment

iii- ALO group

Bone density tended to increase by time with the least value recorded at 2 weeks and the maximum values obtained after 8 weeks. Analysis of variance (ANOVA) test revealed that the difference between groups was statistically significant (p<0.0001). Tukey's post hoc test revealed that the difference between each two intervals is statistically significant (Table 8, Fig.8).

Table (8) Grey scale in ALO group throughout the experiment and statistical significance of the difference using ANOVA test

ALO	2 weeks	4 weeks	6 weeks	8 weeks
Mean	140.75^a	159.75^b	171.00^c	190.63^d
SD	6.39	5.50	5.88	4.37
Min	133.00	152.00	164.00	186.00
Max	151.00	169.00	179.00	199.00
F value	111.81			
P value	<0.0001***			

*** Statistically high significant

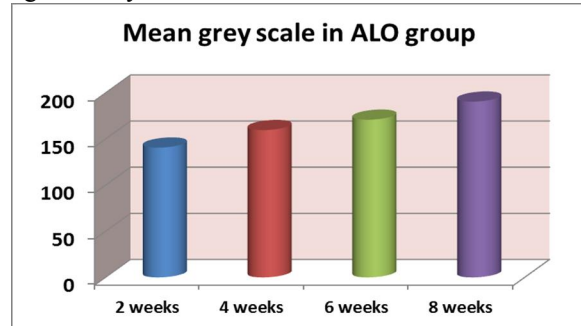-Tukey's post hoc test: means with different letters are significantly different.

Fig (8) Mean grey scale in ALO group throughout the experiment

iv- PRP+ ALO group

Bone density tended to increase by time with the least value recorded at 2 weeks and the maximum values obtained after 8 weeks. Analysis of variance (ANOVA) test revealed that the difference between groups was statistically significant (p<0.0001). Tukey's post hoc test revealed that the difference between each two intervals is statistically significant (Table 9, Fig.9).

Table (9) Grey scale in PRP+ALO group throughout the experiment and statistical significance of the difference using ANOVA test

PRP+ ALO	2 weeks	4 weeks	6 weeks	8 weeks
Mean	145.25^a	167.63^b	177.13^c	192.25^d
SD	4.86	5.18	5.38	5.26
Min	139.00	161.00	169.00	187.00
Max	152.00	177.00	184.00	201.00
F value	115.8			
P value	<0.0001***			

*** Statistically high significant
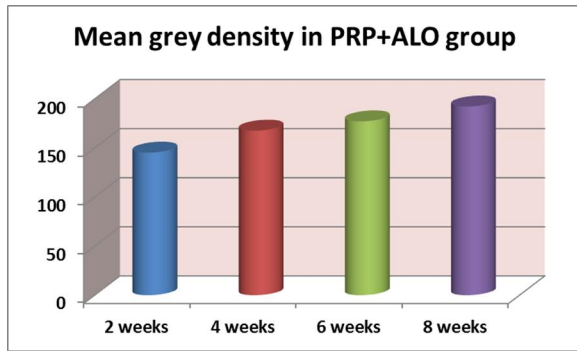-Tukey's post hoc test: means with different letters are significantly different

Fig (9) Mean grey scale in PRP+ALO group throughout the experiment

C- Mean gap distance (µm) after 8 weeks

The greatest mean gap distance (µm) was recorded in the control group, whereas the ALO+PRP group recorded the least distance. Analysis of variance (ANOVA) test revealed that the difference between groups was statistically significant (p<0.0001). Tukey's post hoc test revealed that the difference between ALO group and ALO+PRP was insignificant (Table 10, Fig.10 &11).

Table (10): Gap distance (µm) in different groups at 8 weeks

	C group	PRP group	ALO group	ALO+PRP group
Mean	6.05[b]	3.98[b]	0.77[c]	0.73[d]
SD	0.22	0.19	0.17	0.18
Min	5.52	3.70	0.59	0.57
Max	6.20	4.21	1.03	1.01
F value	1502.93			
P value	<0.0001***			

*** Statistically high significant

-Tukey's post hoc test: means with different letters are significantly different.
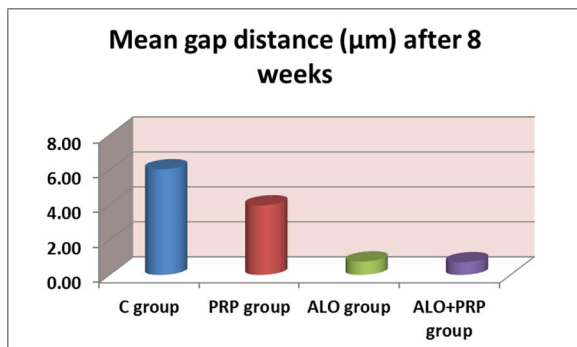

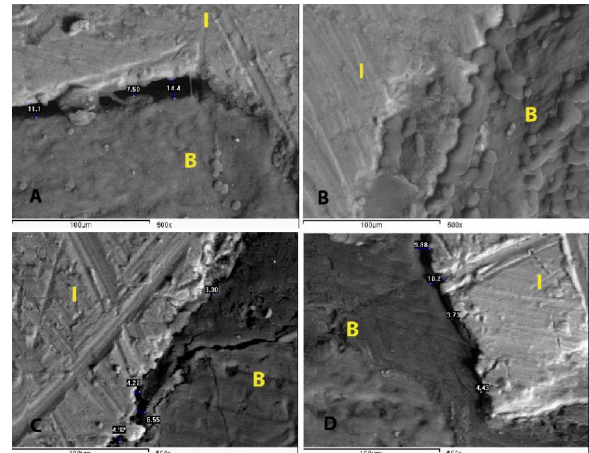Fig. (10) Mean gap distance (µm) in different groups at 8 weeks

Fig. (11) SEM of the four groups at the end of 8 weeks showing gap distance at bone-implant interface (SEM X 500), A. control group, B. ALO+PRP group, C. ALO group and D. PRP group while I represents implant and B yellow represents bone.

4. Discussions

The replacement of missing teeth with endosseous implants for the rehabilitation of edentulous or partially edentulous patients has become a standard of care in the past two decades. This significant progress is based on the osseointegration concept (Brånemark et al., 1969).

Fundamental experimental studies demonstrated that titanium implants regularly heal with direct bone-to-implant contact, a process termed osseointegration (Brånemark et al., 1969) or functional ankylosis (Albrektsson et al., 1981).

To achieve and maintain osseointegration, indications, and contraindications must be carefully balanced, and proper patient selection is thus a key issue in treatment planning. Contraindications classified into local and systemic or medical. Systemic diseases may affect oral tissues by increasing their susceptibility to other diseases or by interfering with healing. In addition, systemic conditions may be treated with medications or other therapies that potentially affect implants and their surrounding tissues (Blanchaert et al., 1998)

Several authors have identified diseases that interfere with dental implantation such as rheumatoid arthritis, osteomalacia, osteogenesis imperfect. In addition, immunocompromised patients (HIV, immunosuppressive medications); drug abusers (alcohol); noncompliant patients(psychological and mental disorders); patients with osteoporosis, irradiated bone (radiotherapy), severe diabetes (especially type 1), bleeding disorders (hemorrhagic diathesis, drug-induced anticoagulation), heavy

smoking habit were also considered (Blanchaert et al., 1998 and Buser et al., 2000)

Osteoporosis is a systemic skeletal disease characterized by reduced bone strength that predisposes to an increased risk of fractures. It is prevalent in females and its incidence increases with age. In addition, it is characterized by a deterioration of bone microarchitecture with reduced bone mass, strength and increased fragility. It has been established the hypothesis that osteoporosis affects the jaws in the same manner as other bones of the skeleton. Thus, altered bone metabolism might induce scarring around the implants (NIH report, 2001). Consequently, osteoporosis represents a systemic risk factor for osseointegration (Praiss, 1986 and Linder et al., 1988).

The most commonly used medications for osteoporosis are the antiresorptive bisphosphonates, which reduce bone resorption by inhibiting osteoclastic activity. Bisphosphonates (alendronate, risedronate, ibandronate and zoledronic acid) are effective in reducing vertebral and invertebral fractures. Alendronate, the first modern FDA-approved bisphosphonate in 1995 which remain in bone and their effect to decrease bone resorption markers (Bauer et al., 2004, Silverman et al., 2007, Reginster et al., 2006 and Watts & Diab, 2010).

PRP is now applied in tissue engineering and can be used in the most varied areas of the dentistry, being applied in periodontal and maxillofacial surgeries. It is a storage vehicle for growth factors, especially (platelet-derived growth factor (PDGF) and transforming growth factor (TGF) (Issa et al., 2007).

At the present study, the bone density results revealed the greatest mean bone density, in group treated with both ALO+PRP, followed by the ALO group, then the PRP group, with the least value for the control group. In all groups, bone density tended to increase by time with the least value recorded at 2 weeks and the maximum values obtained after 8 weeks. The difference between groups was statistically high significant (p<0.0001). Similarly, the result of SEM in ALO+PRP group recorded the least mean gap distance (µm) after 8 weeks.

Although, other researches were not able to clarify the effectiveness of ALO on peri-implant bone formation and hence osseointegration (Nociti et al., 2002 and Frenkel et al., 2001). The favorable results of bone density of the present study could be attributed to combination of ALO with PRP (group IV). It seems that PRP had a synergistic effect on ALO. All previous studies showed that PRP enhances bone regeneration during the first phase of bone healing at implant bone interface (early healing). PRP in the normal bone defect or osteoporotic one has benefits for organizing the formative cell (especially osteoblast), formation of neovascularization and rapid

apposition of bone matrix with its mineralization process. It could supplement healing tissues in animals and both accelerate and potentiate two processes. Firstly local hemostasis at sites of vascular injury and secondly nourishment for undifferentiated cells to be differentiate and provide significant effects for their migration to the healing area as well as activate its biological role (Marx et al., 1998, Anitua, 1999, Rodriguez et al., 2003, Wojtowicz et al., 2003 and Zechner et al., 2003).

In addition, platelets can enhance the plasminogen activation capacity of mesenchymal progenitors, which responsible for bone formative cell. These findings are indicated that the platelets within PRP release growth factors and proteins like osteonectin, fibronectin, and osteocalcin, all of them influence bone healing in different ways also PRP has an osteopromotive activity since it contains a concentrated growth factors that increasing cellular proliferation. These results could be observed by the presence large numbers of haversian canals, which mean that there was increased in blood supply (Agis et al., 2009, Marx, 2004 and Al-Kurikchy et al., 2008)

Another factor may increase bone density in ALO+PRP group, PRP may counteract the action of ALO on vascularization and inhibition of angiogenesis and vascular endothelial growth factor (VEGF) (Fournier et al., 2002). VEGF is a potent cytokine present in PRP and cancellous bone. A strong stimulus of VEGF secretion is hypoxia as recognized in early wound healing where oxygen deprivation occurs and stimulates glycolysis to increase energy production. This cascade of events increases VEGF levels, which may in turn increase vascular permeability and stimulate the formation of new blood vessels and revascularization of the bone matrix and soft tissues (Marx et al., 1998, Anitua, 1999, Chiarotto et al., 1999 and Ladoux and Frelin, 1993).

Furthermore, ALO could increase bone density through its affinity for binding to the mineral matrix of bone. Furthermore, their primary pharmacologic effect is the inhibition of bone resorption by decreasing osteoclastic function. Because of this specific pharmacologic effect, the number of osteoclasts will be increased in osteolytic lesions (Marx et al., 2005, Russell et al., 1999 and Licata, 2005). With increased osteoclastic activity, cellular activity of bone remodeling and resorption is disrupted. The ALO will prevent differentiation into osteoclasts by monocytes and macrophages and will stimulate apoptosis of osteoclasts. With disruption of the osteoblast-osteoclast homeostatic cycle, osteoblast activity remains unaffected, which results in increased bone mass and density (Marx et al., 2005, Ruggiero et al., 2004, Pharmaceutical report, 2004 and Hellstein et al., 2005). Moreover, the ALO drug are not

metabolized and can remain in bone for many years impairing the homeostatic cycle of bone remodeling and repair (Marx et al., 2005, Ruggiero et al., 2004, Pharmaceutical report, 2004 and Hellstein et al., 2005).

Giro et al (2008) in an experimental study stated that; bone density with estrogen privation had a negative impact in the bone and alendronate treatment presented the highest density for all evaluated regions. Similarly, the results of Da Paz et al. (2001) and Rico et al. (1999) 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. Thus, this could explain the improvement of bone quantity and quality showed with ALO+PRP combination than the independent ALO at early stage of healing but the difference was insignificant after 8 weeks of healing. Accordingly, the favorable effect of using ALO was augmented by the PRP during the first phase of healing.

Therefore, it could be suggested that the use of Alendronate in combination with platelet-rich plasma could normalize the high rate of bone turnover that characterizes osteoporosis. Consequently, enhancing the early stability of implants, reserve bone mass around the implant and help surrounding bone growth into the implants' surface, thus promoting efficient implant osseointegration.

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