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Comparative Investigation of The Effects of Platelet-Rich Plasma in Sinus Lifting

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Abstract

Background: *In this study, we aimed to evaluate the produced bone's quality, quantity, volume and the period of the healing process clinically, radiologically, histologically and histomorphologically after a sinus lifting operation using a natural mineralized bone graft with or without Platelet Rich Plasma (PRP).*

Material and methods: *22 sinus elevation operations under local anesthesia performed on 16 patients with no systemic illnesses which may affect healing. Before the operations, orthopantomograph and conical beam 3D dental tomographies of all patients were taken. Ten of the sinus lifting operations were performed by using mineralized hydroxyapatite and PRP mixtures produced from the patients' own blood samples (Group 1); the other 12 sinus lifting operations were performed by using natural mineralized hydroxyapatite (Group 2). Orthopantomograph and computerized tomographies of the first group of patients were taken following a four-month healing period and for the second group following an eight-month healing period. After that, a bone biopsy was taken using trephine burs and dental implants were installed. At the end of the four-month healing period, biopsy samples were taken from two patients in the second group. The samples were evaluated histomorphologically.*

Results: *In the histomorphological evaluation, core biopsies taken from the first group after the fourth month revealed greater bone formation, osteoid tissue and lamellar matrix. But no significant differences were statistically observed between the two groups.*

Conclusion: *Although there were no significant differences in amount of bone healing, the bone healing of the first group was faster than that of the second group.*

Key words: *Platelet-Rich Plasma, Maxillary Sinus, Guided tissue regeneration, Bone regeneration*

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Introduction

Rehabilitation of the edentulous posterior maxilla with dental implants can be a problem because of insufficient bone volume both vertically and horizontally caused by atrophy of the edentulous alveolar crestal bone and pneumatization of the maxillary sinus. Consequently, primary implant stability could not be obtained in this region because of the absence of useful cortical bone and the loose structure of cancellous bone[1].

The first application of maxillary sinus lifting was done by Boyne in order to augment both bone height and volume for prosthetic application in the 1960s[2]. Sinus lifting surgery is a procedure which was developed in the 1980s and it became a new treatment option for dental implants in the maxilla[3]. In less severe cases, when the remaining alveolar bone has a minimum height of 5mm, sinus floor elevation and implant insertion can be performed in one session. In cases of severe atrophy of the maxillary alveolar process, when no good primary stability can be expected, sinus floor elevation and implant insertion are generally performed in two stages[4,5].

Autogenous grafts are considered the gold standard and have some advantages such as osteogenic potential, and they do not generate immune reaction; osteoinductive and osteoconductive potential are preferred in sinus lifting operations[6]. However, because of there are some disadvantages (such as the need for second surgery, morbidity of donor site, insufficient availability, lengthy operation time, increased blood loss and post-operative pain), many other types of materials have been created as substitutes for autologous bone such as allograft, heterograft and alloplastic material. However, none of these substitutes could achieve the level of autogenous graft healing and maturation of these graft materials could take up eight months when used for sinus lifting[7].

Recently, studies have focused on applying growth factors (GF) to increase bone regeneration. These growth factors regulate the cellular events of all the important stages of bone regeneration such as chemotaxis, mitogenesis and differentiation. These GFs were mixed with graft material because they accelerate the process of bone healing[8].

Platelet Rich Plasma (PRP) contains the following GFs: platelet-derived growth factor (PDGF), transforming growth factor-b (TGF-b), vascular endothelial growth factor (VEGF), epithelial growth factor (EGF), insulin growth factor-1 (IGF-1), basic fibroblast growth factor (bFGF), as well as three blood proteins known to act as cell-adhesion molecules for osteoconduction (i.e., fibrin, fibronectin and vitronectin)[9,10]. The basic hypothesis of PRP addition to bone grafts is that high concentrations of platelets in a bony wound will increase the local concentration of secreted growth factors and subsequently enhance the initial bone-healing response. Later on, the direct influence of PRP will fade away and the physiological mechanisms of bone repair will continue to work at an accelerated level[11].

The aim of this clinical and histological investigation is to evaluate the effects of PRP on bone healing in sinus lifting surgery.

Materials and Methods

Ethical statement

The study was conducted in accordance with the standards of the Declaration of Helsinki of 1983. This study's procedures were approved by the Ethical Committee of the Dicle University Faculty of Dentistry (Process No: D.Ü.D.F.E.K. 2008/0006821).

Patient Selection

This study comprises 22 cases of sinus augmentation performed natural mineralized hydroxylapatite (HA, Apatos®, Tecnoss, Italy) with or without PRP (Table.1). The patients were informed about the possible complications of the operation, as well as the aim and design of the study and written consent was obtained.

Groups/Material	HA+PRP	HA	HA
Group 1	10 specimen, 4month		
Group 2		10 specimen, 8 month	2 specimen 4 month

HA:hydroxylapatite, PRP:Plasma rich platelets
Table 1. Organization of Patients and Groups

The study sample comprised 16 patients (5 women and 11 men) with a mean age of 43 years (range 31 to 64 years) referred to the Department of Oral and Maxillofacial Surgery, Dicle University Faculty of Dentistry, Diyarbakir, Turkey for implant treatment. All patients were candidates for augmentation in the posterior maxilla prior to implant surgery. One surgeon performed all the surgical procedures.

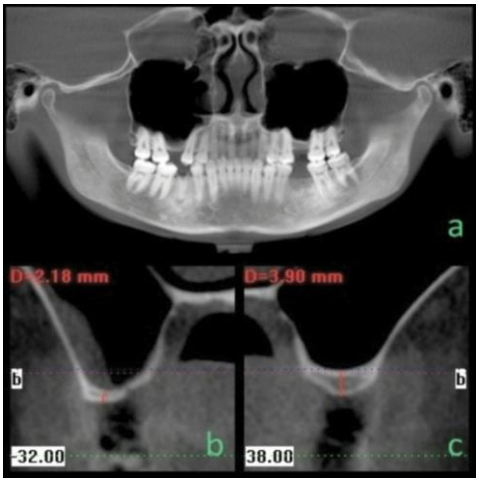

Figure 1: *Pre-operative cone beam computerized tomography scan*

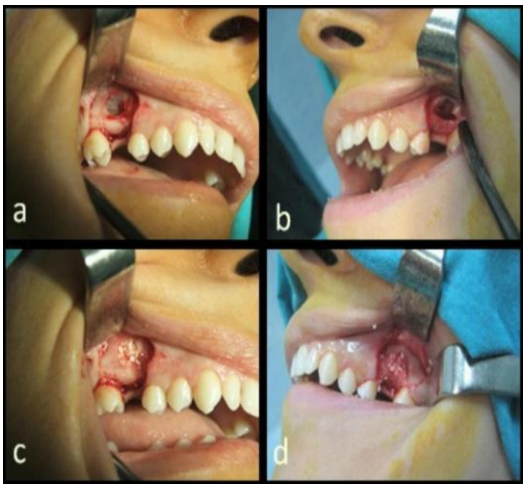

Figure 2: *Clinical view of maxillary sinus lift with HA and HA+PRP*

Patients with immunologic diseases, unstable diabetes mellitus, ongoing chemo- or radiotherapy were excluded. The inclusion criteria were an absence of a history of maxillary sinus inflammation. Clinical examination and preoperative radiographs showed a severe atrophy of the maxilla (less than 5 mm residual bone) diagnosed by panoramic radiographs and by cone beam tomography (Fig. 1).

Fifteen of the patients were partially edentulous and one was totally edentulous. All patients were scheduled to receive fixed prostheses or crown restorations. Smoking was not regarded as an exclusion criterion, but smokers were advised to refrain from smoking or to reduce their smoking.

Preparation of PRP

One hour before surgery, 12 ml of blood was drawn into 3 citrated tubes. Tubes were centrifuged for 10 minutes at 1000 rpm (CS-15 Centrifuge, no: 96 E 6773,4800 rpm) at Dicle University, Medical Faculty, Hematology Laboratory. This procedure divided the blood into three basic layers: erythrocytes at the bottom of the tube, and plasma rich and platelet poor plasma (PPP) at the top of the tube. The upper part of the tubes and the upper 1 to 2 mm of the red blood phase were collected and centrifuged for 10 minutes at 2000 rpm to concentrate the platelets. The PPP was removed in order that the PRP remained in the tube. The volume of the cell-free plasma was reduced to approximately 1 ml. The PRP obtained was activated by CaCl solutions.

Surgical procedure

All 22 sinus lifting operations were done according to the lateral approach technique, of these, 8 were unilateral, and 7 were bilateral. All patients were treated under local anesthesia; approximately 4 ml (4%) articain HCl with epinephrine HCl (1:200,000) (Ultracain D-S Fort, Sanofi-Aventis Deutschland GmbH, Germany) was used for each patient. All patients were also given prophylactic antibiotics (ampicillin+sulbactam), 750 mg, twice daily; 1 day before surgery and after surgery for 7 days.

After a crestal incision with a vertical releasing incision, a mucoperiostal flap was elevated and reflected laterally to expose the lateral wall of the sinus. Osteotomy was performed on the lateral wall of the maxillary sinus with a round bur. The bone in the centre of the window was left attached to the schneiderian membrane. Care was taken not to lacerate the sinus membrane and the sinus mucosa was elevated using a sinus elevation kit. The subantral space was grafted with natural mineralized hydroxyapatite (HA, Apatos®, Tecnoss, Italy) mixed with PRP in group-I (10 region) and only HA in goup-II (12 region) (Fig. 2). The graft mixture was packed layer by layer. An absorbable collagen membrane (Osteobiol®, Tecnoss, Italy) was then placed on the vestibular wall of the sinus. The wounds were primarily sutured.

Harvesting of the bone specimen

Bone harvesting was performed when the implant insertion was done four months following sinus lifting for group I and eight months for group II after the cone beam CT was taken (two of them were harvested at fourth months) (Fig. 3). Core biopsies were obtained by 2 mm trephine burs before using implant drills, but to guarantee that the augmented region of interest was examined, a drill was used before the trephine bur to eliminate superficial and non-regenerated bone (Fig. 4). The biopsy materials were stored in 10% formalin and sent to Dicle University, Medical Faculty, Histology and Embryology Department.

Osteogenesis absent	1
Osteogenesis poor	2
Osteogenesis moderate	3
Osteogenesis good	4
Osteogenesis excellent	5

Table 2. *Scores of histological evaluation*

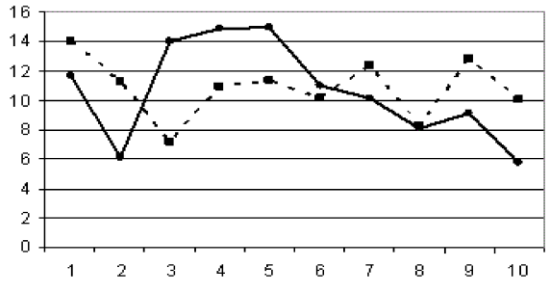

Table 3. *Graphic of b*one height obtained after sinus lifting (- Group I, - - Group II).

Histomorphological examination

Biopsy materials were fixed in formaldehyde solution decalcified with 5% nitric acid for 5 days. After that, they were dehydrated in alcohol and embedded paraffin and 5 µm sections were made from paraffin blocks using microtome. Paraffin sections were stained according to 3 protocols: Hemotoxylin-Eosin (H-E), Masson Trichrome and Van Gieson. The slides were then examined under a light microscope (Olympus BH-2). The presence of newly formed bone was scored from 1 to 5 by an expert (Table 2). All samples were examined by randomized expert.

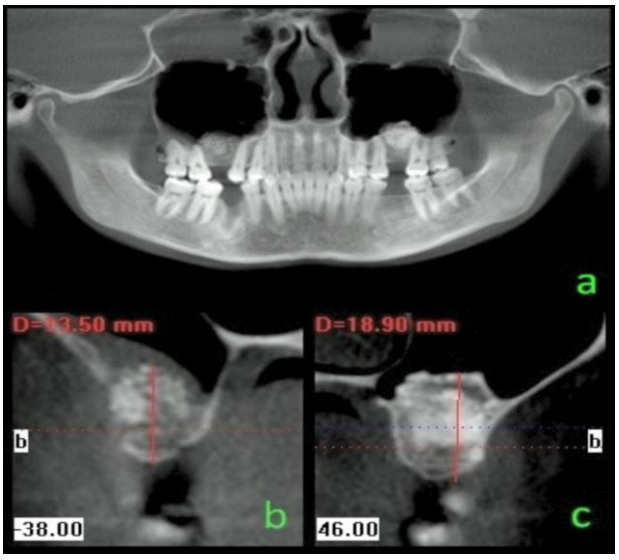

Figure 3: *Cross section of scans of the sinus floor after healing period*

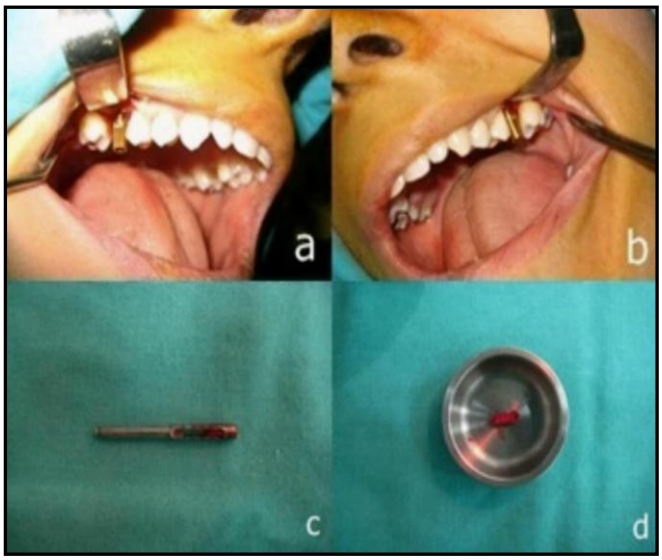

Figure 4: *Harvesting of core biopsy during implant insertion*

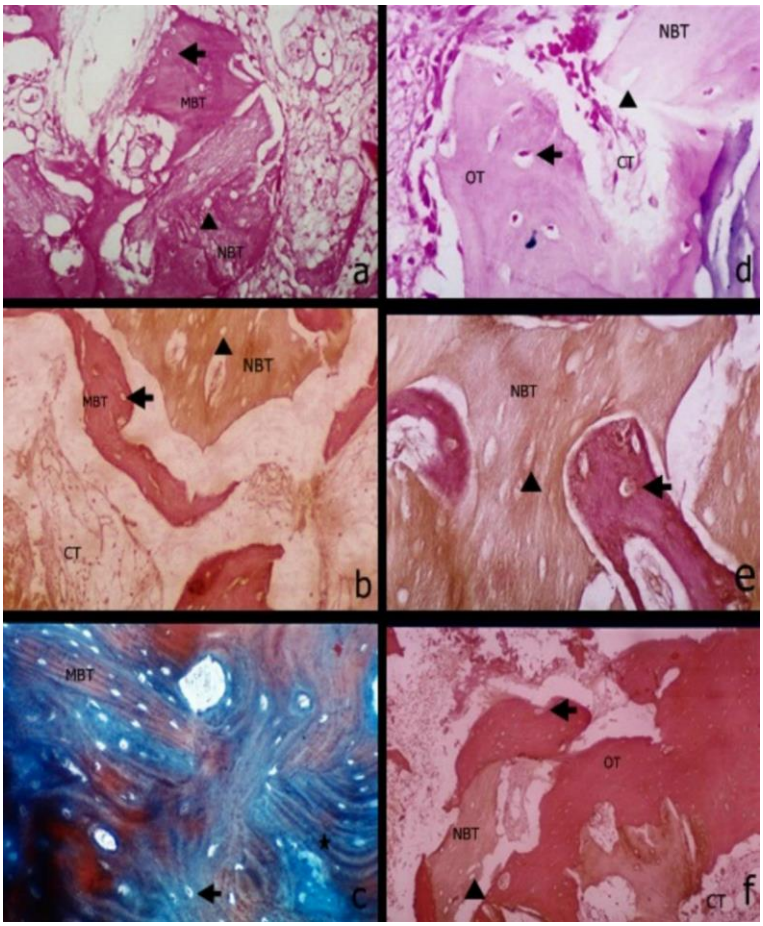

Figure 5: The trabeculae of the mineralized and necrotizing bone tissue is observed together in NMHA+PRP group sections obtained after 4 months (a, b, c). In addition to the mineralized and necrotizing bone tissues, the presence of connective and osteoid tissue is seen in NMHA treated group sections obtained after 8 months (d, e, f). MBT: mineralized bone tissue, arrows: osteocyte-containing lacunas, arrowhead: lacunas lacking osteocytes, NBT: necrotizing bone tissue, OD: osteoid tissue, CT: connective tissue, asterisk: bone lamellae (a, d: Hematoxylene and Eosin, b, e: Van Gieson, c, f: Masson trichrome staining, original magnification, b, f: X40, a, c, d: X80, e: X160).

Clinical and Radiological Examination

During surgery and postoperative complications, soft tissue and bone tissue healing and infection were evaluated clinically. Panoramic radiographs and Cone Beam 3D Dental Tomography (i-CAT®, USA) were obtained before operations and after the healing periods of each group. Bone heights obtained after surgery were evaluated.

Statistical analysis

Statistical analysis was performed using a commercially available software program (SPSS 14.0, SPSS Inc., Chicago, IL, USA). Median values are given as descriptive statistical values for discrete variables in statistical analysis.

Mean values and standard deviations were calculated for the amount of regenerated bone after 4 and 8 months of healing. The data were subjected to statistical analysis with the Mann–Whitney U-test. Differences of $P < 0.05$ were considered significant.

Result

Clinical and radiographical findings

There was 1 perforation (4%) at the schneiderian membrane during the elevation procedure. The perforation was covered with resorbable collagen membrane and the operation was carried out; this did not seem to complicate the healing.

During and after the operations, bleeding problems did not occur; however, it was observed at the sinus lifting operations that vascularity was better on patients with teeth.

The results of CBCT pre- and post-operative measurements are shown in Table 3. On average, 10.6 mm (5.76mm-14.85mm) bone height was provided in the 22 sinus lifting operations. It was observed that there was enough bone in order to make implants in the maxillary sinus lifting operation by using

HA and HA + PRP. It was observed that during the operation, grafts were more easily manipulated by mixing PRP with the graft material. In the operations where only HA was used, it was observed that particles were scattered during grafts being applied to the cavity. Due to this fact, it was thought that PRP combined graft pieces to each other.

Histological analysis

Regularly arranged bone lamellae and lacunae with osteocyte were observed in the newly formed bone tissue in slides stained with H-E in Group I. Bone lamellae were observed as thin and long structures. Soft tissue filling the space between the bone trabeculae was observed, as well as connective tissue containing fibroblasts, collagen, and blood vessels (Fig 5a). Newly formed mineralized bone trabeculas (MBT) were stained in dark red although necrotic bone trabeculas (NBT) were stained in dirty yellow on slides stained with van Gieson stain technique in Group I. It was observed that lacunas were empty in NBT; however lacunas had osteocyte in MBT (Fig 5b). Bone lamellas showed an orderly sequence on the new bone trabeculas on slides stained using the Masson trichrome stain technique for the same group and it was observed that MBT stained in shades of blue with Masson trichrome (Fig. 5c).

Similar observations to Group I were obtained from the histological findings by light microscopy on slides stained with H-E and van Gieson in Group II (Fig. 5d, e.). However there was a different view on slides stained using Masson trichrome for this group. Although NBT partially protected the existence, there was some mineralized tissue formation on the periphery of the trabeculas which were stained in blue for this reason. There was an important finding that the majority of the other trabeculas should be stained in blue; however, they stained in brick-red. These trabeculas had not calcified and were at the newly osteoid tissue stage (Fig 5f).

It was observed that the development of bony tissue has not been completed and more time was needed for recovery on the slides stained with H-E slides of two patients at fourth months using only HA. The median of Groups I and II were calculated as 3 and the median values of the two groups were compared using the Mann-Whitney U test. No significant difference was found between the two groups (Table 4) (p=0,990).

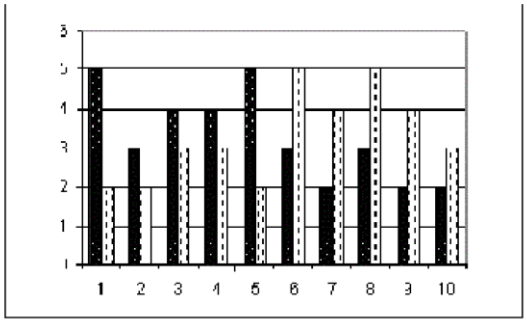

Table 4: *Statistical findings of formed bony tissues*

Discussion

Use of dental implants has become a routine application in oral surgery. The maxillary sinus causes an anatomical limitation in the reconstruction of the edentulous maxillary posterior area. Therefore, the existing bone may not be enough to allow for the placing of implants. In order to overcome these limitations, a sinus lifting operation has been developed[12-20]. Various methods have been developed for the maxillary sinus lifting operation. In much of the literature, there is consensus on the surgical method which is applied by using a lateral

entry window[17-19]. In our study, by using HA and HA+ PRP on patients with resorbed alveolar crest, pneumatized maxillary sinus and have an edentulous maxillary posterior region, we carried out maxillary sinus lifting operations and we compared the healing of these two groups. We observed that the sinus lifting operation is a very effective way to enhance the volume of bone in the edentulous maxillary posterior area, as per previous studies[21].

There has not been a consensus about the graft materials, and most researchers agree that either mixed bone grafts or autogenous grafts is the best option[17-19]. A second operation is required for autogenous bone grafts which increases operation processes, possible complications and patient morbidities. Although the first studies suggest using only autogenous bone grafts in sinus lifting operations, tricalcium phosphates, bovine cancellous grafts and allografts have been developed in order to eliminate these disadvantages[8,19,21-26]. Xenografts taken from different kinds of beings have been used, including bovine bone minerals (BBM) and porous HA which is obtained from coral skeletons. The mineral structure and surface of BBM resembles autogenous bones and because of the way it behaves, it is a suitable osteoconductive material[23]. While osteoconductive grafts (e.g. collagen preparations, muted bone, ceramics and spongiosa bone) serve as a guide to the formation of the new bone which starts form the bone, osteoinductive grafts (demineralized bone matrix) cause the morphogenesis, organogenesis and heterotopical union at the new bone formation[27]. So, in oral and maxillofacial surgery, researchers deal with developing bone graft techniques and providing faster and more intense bone regeneration. Therefore, some studies report that using growth factors is a real way to both enhance and speed up soft tissue and bone healing[28]. However, Velich et al. (2004) used autogenous bone, heterograft, exogenous bone and synthetic material in sinus lifting operations by alone or by mixing growth factors in their study. With these materials, they could not find a different result, apart from the gel calcium carbonate which has a high absorption feature in its formation[29].

Preclinical studies have proven that platelets have growth factors which stimulate and enhance bone healing[12,30]. The studies carried out on accelerated healing have focused on those factors which can enhance bone formation following the guided tissue regeneration (GTR) technique or the GTR technique combined with graft materials. Current studies have proven that when PRP (which has an excessive peptide growth factor level) is used with autogenous and heterogenous materials, it will enhance new bone formation[12,24].

In an experimental study, Aghaloo et al. (2002) filled the defects that they had created in 15 rabbits' craniums. They voided one of the 4 defects and filled the others with autogenous bone, autogenous bone + PRP and only PRP. At the histological assessment, more bone formation was observed in the defects which used autogenous bone and PRP + bone. At the histomorphometric observation, more bone area was found on the defect which had been applied with bone+ PRP[28]. Pryor et al. (2005) created cranial defects in 30 rats and filled 18 of them with absorbable collagen sponge (ACS) and PRP and 12 of them with only ACS. They observed that bone formation occurred at an extremely high rate in the regions where PRP had been used[31].

After graft materials have been placed on the subantral space, grafts are fed only through diffusion and vascularised after some days or weeks. When there is no vascular support of the graft, most grafted osteoblasts and precursor cells die after a long period. In this way, bone purifies corner cells and osteoclasts become active. On one hand, these results with a reduction in biomechanical stability, but on the other hand, new bone formation becomes possible with an increased amount of calcium[2].

Kassolis et al. (2005) stated that they achieved a good result through the combination of freeze-dried bone allograft (FDBA) with PRP at subantral sinus lifting. In the same study, it was observed that PRP with FDBA enhances bone formation at sinus grafting. However, they stated that they need to undertake further studies in order to ensure the clinical effects of the increase in bone formation[32].

Marx et al. (1998) showed that bone graft cells need to keep the receptors of growing factors in platelets. Radiographies and computerized tomographies showed that the bone mineral density of the grafts supported with PRP is 1.6–2.2 times greater than for grafts that were unsupported with PRP. This increase is a sign of bone formation more quickly and maturing earlier of the bone graft stimulated with PRP clinically. Histhomorphometric studies showed that while autogenous bone grafts without PRP produce 55% ± 8% volume bone trabecule, PRP-supported bone grafts produce 74% ± 11% volume bone trabecule. This measurement is a sign of the increase in bone density produced by PRP[33].

Kassolis et al. (2000) showed that the combination of FDBA and PRP provided a good cure in sinus lifting both clinically and histhologically. Surgically, the use of FDBA allogenic bone replacement grafts eliminates the need to take autogenous bone. Although the degree of bone regeneration following FDBA use is generally acceptable in the alveolar and sinus lifting process, its capability for increasing bone healing and regenerative quality must be developed in order to provide a decreased healing period. They stated that further studies were necessary in order to decide whether PRP increases new bone formation or not[12].

Eun-Seok et al. (2001) created bone defects in 20 New Zealand rabbits calvaria. They filled 10 of them with natural cancellous bovine bone mineral and PRP mixture and they filled the other 10 with only natural cancellous bovine bone mineral. At the radiographic assessment, it was reported that the mineralization rate in the test group was 54.7% ± 5.9% after 4 weeks; it was 77.4% ± 4.9% after 8 weeks. The control group rate was reported as 38.3% ± 6.5% after 4 weeks and it was 51.0% ± 4.0% after 8 weeks[34]. Schlegel et al. (2007) carried out 48 sinus lifting operations using a lateral approach by pulling out 24 pigs premolar teeth. In this operation, they used autogenous graft, autogenous graft + PRP, Bovine HA, Bovine HA + PRP. As a result, they reported that mixing autogenous and Bovine HA graft with PRP had not made any difference[35]. Choukroun et al. (2003) achieved sinus lifting operations in nine regions and they applied FDBG combined with PRF in six cases and applied FDBG only in three cases. In the histimorphometrical observations of the control group (FDBG) after eight months and of the test group (PRF + FDBG) after four months, they reported that they did not observe any difference[7]. In our study, it was observed that there was more osteoid tissue development and mineralization in the samples we took from the HA+ PRP group at the fourth month compared to the HA only group at the eighth month.

Froum et al. (2002) published the histological results of three patients who had undergone bilateral sinus lifting operations. One region of the patients' sinus was filled with a mixture of inorganic bovine graft and PRP and the sinus on the other region was filled only with inorganic bovine graft. On one patient, inorganic bovine graft was mixed with autogenous graft. In this comparative study, it was reported that PRP has no benefit on the production of vital bone[15]. However, Kassolis et al. (2005) carried out bilateral sinus lifting operations on ten patients in their study. They used FDBA and closed the bone window with resorbable membrane in the control group and they used a mixture of FDBA + PRP and closed the bone window with PRP membrane in the experimental group. The implants were applied after four, five and six months after the grafting operation. They reported that the rate of live

tissue (78.8% ± 8) in the histhomorphometric analysis was greater in the experimental group where FDBA + PRP had been applied. They also reported that the rate of bone development (33.3% ± 11.3%) in the experimental group where they used FDBA + PRP was not so different from the control group (26.5% ± 6.8%) which used FDBA + membrane. They showed that the amount of the rest graft was greater for the control group (37.0% ± 15.7%) that used FDBA + membrane compared with the experimental group (21.2% ± 8.3%) that used FDBA + PRP. The study showed that at the sinus lifting operation, PRP increases osteoconductive activity in the nature of FDBA[32].

According to information taken from endoscopic investigations and sinus membrane biopsy examples of patients whose sinus membrane was damaged during implant placing, it was reported that the sinus membrane did not show any morphological change under the light microscope; there was no change in the columnar silver epithelium and mucus production of secretional goblet cells, although sometimes unknown matter may migrate through the sinus membrane[6]. Raghoebar et al. (2000) reported that perforation occurred at the rate of 26% of sinus membranes in their study[36]. Jensen et al. carried out 128 sinus lifting operations and 34 nasal lifting operations on 98 patients in their study. They reported that sinus membrane perforation occurred in 45 patients (35%). Intraoperatively, a serious bleeding problem was observed in 18 cases (28%) out of 65 partially edentulous patients undergoing sinus lifting operations and 4 cases (6%) out of 63 edentulous patients undergoing sinus lifting operations. Researchers connect the reason for greater bleeding potential from toothed jaws to the fact of their having greater vascularization than edentulous jaws[37]. In our study, there was only 1 (4%) sinus membrane perforation out of 22 sinus lifting operations.

It was determined from the samples which were taken after eight months from the patients who had HA applied in the maxillary sinus lifting operation, that new formed bone trabecules are composed of osteoid tissue, and the lamellous structure hadn't been completely formed in our study. On the other hand, the samples which were taken after four months from the patients who had HA+PRP applied, calcification and lamellation of new formed bone trabecules is in good order. In other words, mature bone has been properly formed. Briefly, it is concluded that PRP application accelerates mature bone formation and shortens the healing period following grafting. Furthermore, the need to take blood from the patient for the application of PRP, plus the time needed for preparing PRP under laboratory conditions prolongs preparation time for the operation.

Conclusion

It was histologically observed that ossification levels in the fourth month following sinus lifting operations using HA grafts applied by mixing with PRP was better than ossification levels in the eighth month after sinus lifting operations using HA grafts only. However, in the statistical analysis of ossification levels, it was determined that there was no significant difference.

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