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Volumetric Comparison of New Bone Formation in Sinus Floor Augmentation Using Allografts with/without Platelet-Rich Fibrin Cohort Research (Retrospective)

Trombositten Zengin Fibrin İçeren/İçermeyen Allogreftler ile Sinüs Tabanı Ögmentasyonunda Yeni Kemik Oluşumunun Hacimsel Karşılaştırması Kohort Araştırması (Retrospektif)

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ABSTRACT Objective: To compare the new bone regeneration potential of bone allograft and bone allograft and platelet-rich fibrin (PRF) mixture with volumetric analysis in sinus floor augmentation. **Material and Methods:** Patients with chronic metabolic disease, smoking and not paying much attention to oral care were excluded from the study. Patients were allocated into two groups. In Group 1, sinus floor augmentations were performed with bone allografts alone and in Group 2, a mixture of bone allograft and PRF was used as sinus bone graft. Graft volume was determined by subtracting the preoperative total sinus volume from the postoperative total sinus volume using the Dolphin programme. Residual and post-operative ridge heights were measured by Simplant software programme on the mid-sagittal section of pre-operative and post-operative cone beam computed tomography (CBCT) images. The mean Hounsfield unit (HU) values were also measured in sagittal sections of CBCT data. **Results:** Thirty patients with 43 sinus floor augmentations were included in the study. The new bone formation and mean HU value in Group 2 were significantly higher than in Group 1 ($p<0.05$). A moderate positive correlation was observed between residual and post-operative ridge height in Group 1 ($p<0.05$; $r=0,048$), whereas there was no significant relationship between residual and post-operative ridge height in Group 2 ($p>0.05$). **Conclusion:** PRF and bone allograft combination may enhance higher levels of new bone formation than bone allograft alone for sinus floor augmentation. The mixture of bone allograft and PRF provides new bone regeneration of sinus floor augmentation regardless of the residual ridge height.

Keywords: Allografts; dental implants; sinus floor augmentation; platelet-rich fibrin

ÖZET Amaç: Sinüs taban ögmentasyonunda kemik allografti ile kemik allogreft ve trombositten zengin fibrin [platelet-rich fibrin (PRF)] karışımının yeni kemik rejenerasyon potansiyelinin volümetrik analizle karşılaştırılmasıdır. **Gereç ve Yöntemler:** Kronik metabolik hastalığı olan, sigara içen ve ağız bakımına fazla dikkat etmeyen hastalar çalışma dışı bırakıldı. Hastalar iki gruba ayrıldı. Grup 1'de sinüs tabanı ögmentasyonları sadece kemik allogreftleri ile yapıldı ve Grup 2'deki sinüs ögmentasyonunda kemik grefti olarak kemik allogreft ve PRF karışımı kullanıldı. Greft hacmi Dolphin programı kullanılarak ameliyat öncesi tam sinüs hacminin, ameliyat sonrası tam sinüs hacminden çıkarılmasıyla belirlendi. Ameliyat öncesi ve sonrası konik ışınlı bilgisayarlı tomografi (KIBT) görüntülerinin orta sagittal kesitlerinde rezidüel kemik ve ameliyat sonrası oluşan yeni kemik yükseklikleri Simplant yazılım programı ile ölçüldü. Ortalama "Hounsfield unit (HU)" değerleri, KIBT verilerinin sagittal kesitlerinde de ölçüldü. **Bulgular:** Kırk üç sinüs tabanı ögmentasyonu yapılan 30 hasta çalışmaya dâhil edildi. Grup 2'de yeni kemik oluşumu ve ortalama HU değeri Grup 1'e göre anlamlı olarak yüksek bulundu ($p<0,05$). Grup 1'de rezidüel ve postoperatif kemik yüksekliği arasında orta düzeyde pozitif korelasyon gözlenirken ($p<0,05$; $r=0,048$), Grup 2'de rezidüel ve postoperatif kemik yüksekliği arasında anlamlı bir ilişki bulunmadı ($p>0,05$). **Sonuç:** PRF ve allogreft karışımı, sinüs tabanı ögmentasyonu için tek başına kemik allogreftinden daha yüksek seviyelerde yeni kemik oluşumunu artırabilir. Allogreft ve PRF karışımı, rezidüel sırt yüksekliğinden bağımsız olarak sinüs tabanı ögmentasyonunda yeni kemik rejenerasyonu sağlar.

Anahtar Kelimeler: Allogreftler; diş implantları; sinüs tabanı yükseltmesi; trombositten zengin fibrin

Maxillary sinus augmentation is a surgical technique that is performed by filling bone substitutes into a three-walled empty room created by the elevation of the Schneiderian membrane with delicate membrane elevators through a laterally osteotomized bone window on the maxillary posterior region in the

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presence of vertical bone deficiency. A variety of bone substitutes of autogenic, allogeneic, xenogeneic, or alloplastic origin can be used as sinus bone grafts. Bone allografts are biocompatible human-derived bone grafts that are available in mineralized or demineralized forms and can be successfully used for sinus floor augmentation procedures.[1] The histomorphometric analysis after sinus floor augmentation with freeze-dried bone allograft (FDBA) revealed better results in terms of new bone formation and residual graft material than inorganic bovine bone, equine-derived bone, and microporous biphasic calcium phosphate graft.[2]

Platelet-rich fibrin (PRF) is a cost-effective blood derivative that can release growth factors and anti-inflammatory cytokines in the related environment and plays a role in the differentiation and proliferation of osteoblasts.[3] PRF is commonly used as an adjunct to bone substitute materials due to its increased healing properties and osteogenic capacity.[4] The mixture of PRF and other types of bone substitutes such as allografts, porous hydroxyapatite, and bioactive ceramics were used in intra-bony defects and ridge or socket preservation procedures.[5,6] Oliveira et al. reported that PRF and inorganic bovine bone mixture had a better bone regeneration effect than PRF alone, inorganic bone alone, and a blood clot in critical-size defects in rats.[7] PRF was used as an adjunct generally to the xenogeneic bovine bone in sinus floor augmentations.[8,9] However, there is only one report that focuses on the results of PRF adjunction to bone allografts in sinus floor augmentation.[10] The null hypothesis was the presence of no difference between new bone regeneration obtained with bone allograft alone and the mixture of bone allograft and PRF in the sinus floor augmentation. This study aimed to compare the bone regeneration potential of demineralized cortical-cancellous bone allograft and the mixture of FDBA and PRF using volumetric analysis of sinus floor augmentation.

MATERIAL AND METHODS

PATIENTS

The retrospective study was approved by the Eskişehir Osmangazi University Non-Invasive Clinical Research Ethics Committee with the approval number E-25403353-050.99-122031 and date December 15, 2020 and was performed in accordance with the ethical standards specified in the Declaration of Helsinki. The data of patients who underwent implant-supported fixed prosthesis to the posterior region after maxillary sinus floor augmentation between April 1, 2013 and March 1, 2018 were obtained from the archives.

Data in undistorted Digital Imaging and Communications in Medicine (DICOM) format, obtained from cone beam computed tomography (CBCT) before and 6 months after the operation, were included in the study. Patients with chronic metabolic disease (diabetes, hypertension, etc.), smoking and not pay more attention to oral care were excluded from the study.

Patients were allocated into two groups. In Group 1, sinus floor augmentations were performed with demineralized bone allograft alone; and in Group 2, a mixture of FDBA and PRF was used for maxillary sinus augmentation.

PRF PREPARATION

Blood was collected with a vascular cannula to be transferred to 10 mL dry tubes containing no anticoagulant. After centrifuging at 2.700 rpm for 12 minutes, the whitish yellowish PRF clot was removed from the tube with a dental tweezer. Then, red blood cells were separated from the PRF with tissue scissors. The PRF was cut into small pieces with scissors and mixed with 2 mL of particulate FDBA (Maxxeus, Community Tissue Services, Ohio, USA) (Figure 1).

MAXILLARY SINUS AUGMENTATION AND IMPLANT PLACEMENT

Lateral osteotomy technique was performed by a single researcher (ÖD) in maxillary sinus augmentation. After performing a rectangular osteotomy of the bone with piezosurgery (Mectron Piezosurgery Device, Mectron, Genova, Italy), the bone in the osteotomy area was gently removed. Elevation was performed with sinus elevators until the Schneiderian membrane was released from the sinus floor (Figure 2). Only freeze-dried cortico-cancellous bone allograft and freeze-dried cortico-cancellous bone allograft mixed with PRF were transferred to the prepared cavity. Only freeze-dried cor-

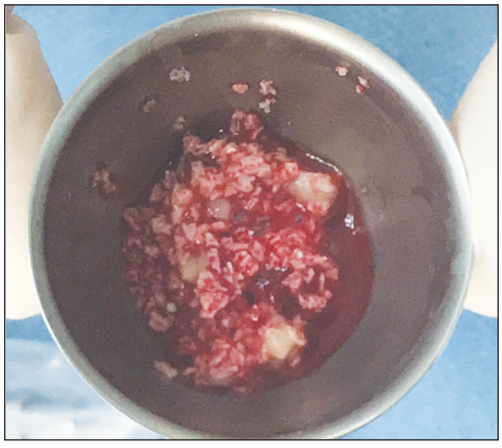

FIGURE 1: Demineralized corticocancellous bone allograft and platelet-rich fibrin fragments were mixed in sterile conditions immediately before application.

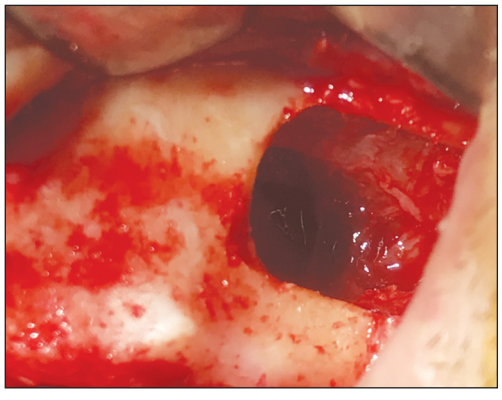

FIGURE 2: The Schneiderian membrane was elevated to the upper boundary of the osteotomy window.

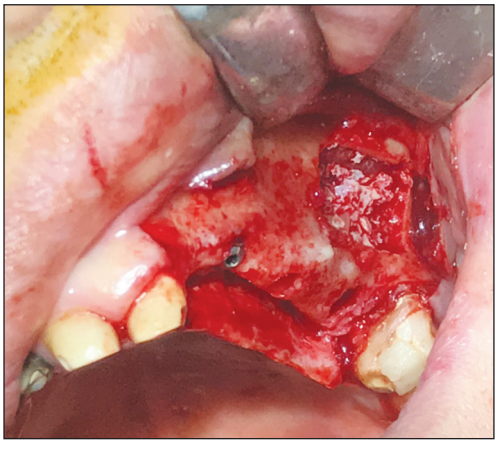

FIGURE 3: The cavity was filled with the mixture of demineralized corticocancellous bone allograft and platelet-rich fibrin after membrane elevation.

tico-cancellous bone allograft and freeze-dried corticocancellous bone allograft mixed with PRF were transferred to the prepared cavity (Figure 3) .Osseospeed TX (Dentsply Implants, Mölndal, Sweden) dental implants with TiO2 sprayed fluoride modified surface (OSTX) were placed under physiological saline irrigation according to the manufacturer's company instructions. The removed bone window was gently placed on the osteotomy site and covered with the membrane obtained by compression of the PRF. The flaps were sutured with resorbable 3-0 vycril sutures. Antibiotics, non-steroidal anti-inflammatory drugs and oral antiseptics were prescribed to the patients postoperatively. Sutures were removed on the 10th day after surgery.

VOLUME MEASUREMENT

In order to avoid bias in the study, CBCT examination was performed retrospectively by the physician (MU) who did not know which procedure was performed on which region. After the data obtained with CBCT were converted to DICOM format, DICOM files were transferred to the software programs to be measured. The subtraction method was used in the cephalometric tracing program of Dolphin 9.0 (Dolphin Imaging, Chatsworth, CA, USA) to measure the total graft volume. In this method, the graft volume was determined by subtracting the complete sinus volume before the operation from the complete sinus volume after the operation. The sinus cavity was marked on sagittal, axial and frontal CBCT sections (Figure 4) and its boundaries were determined. The volume of the sinus cavity was then measured by selecting the measure volume tab (Figure 5). The volume of the marked cavity was displayed with the

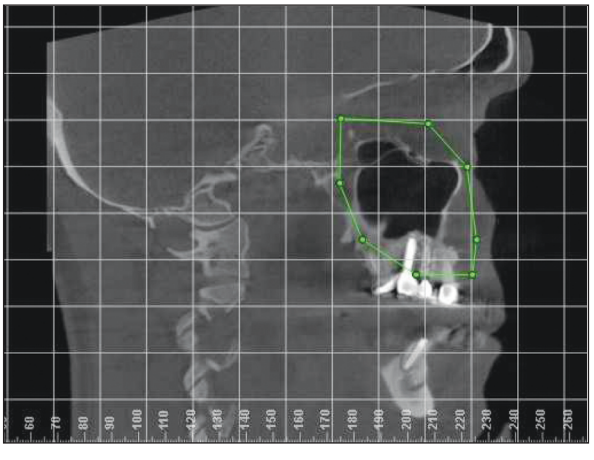

FIGURE 4: Green guidance lines were placed around the sinus cavity on the sagittal section.

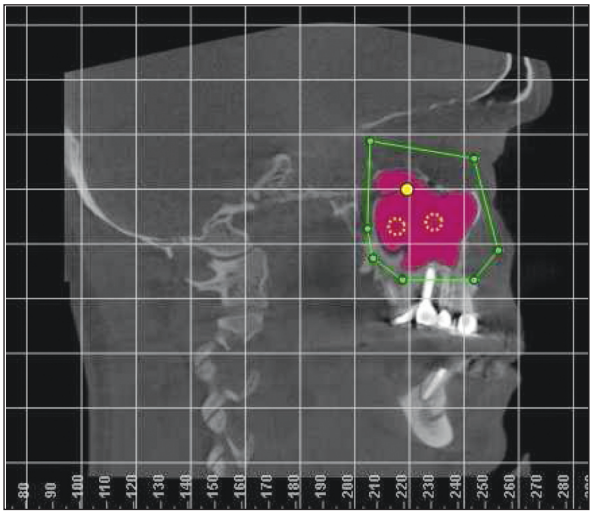

FIGURE 5: The pink area that was formed by the guidance of yellow dots shows the total volume of the air-filled sinus cavity in the sagittal section of a post-operative cone beam computerized tomography data.

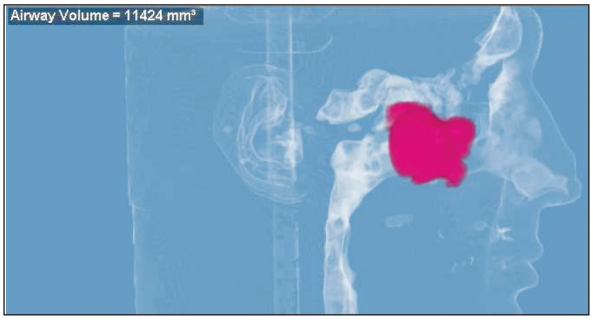

FIGURE 6: The volumetric reconstruction was rendered after volumetric measurement with the guidance of the software program.

extraction feature of the software program (Figure 6). Simplant O&O (Materialise, Leuven, Belgium) tracing software was used to measure the preoperative and post-operative ridge heights on the mid-sagittal section in the central region of sinus floor augmentation (Figure 7).

Hounsfield unit (HU) measurement was also performed to evaluate the bone quality on sagittal sections of CBCT scans as proposed by Martinez et al.[11] The area of the greatest volume of the bone grafts was determined in a sagittal section. Three measurements with 2 mm vertical distance were made in anterior, middle, and posterior regions of newly regenerated bone mass. A total of 9 measurements of made for groups and an average value was calculated for each patient (Figure 8).

STATISTICAL ANALYSIS

SPSS 21.0 package program (IBM, Chicago, USA) was used for statistical analysis of the results. It was determined by Kolmogorov-Smirnov test ($p>0.05$) that the measurement values were normally distributed. The difference in new bone volume and HU value between groups were analyzed with independent samples t-test. Pearson correlation coefficient was used in the analysis of the relationship between residual ridge height and post-operative ridge height.

RESULTS

The mean age of patients was 47.9±9.5. Thirty patients were included in the study and 43 sinus floor augmentations were performed. There were 20 sinus augmentations in Group 1 and 23 cases in Group 2. Ninety-four dental implants were placed simultane-

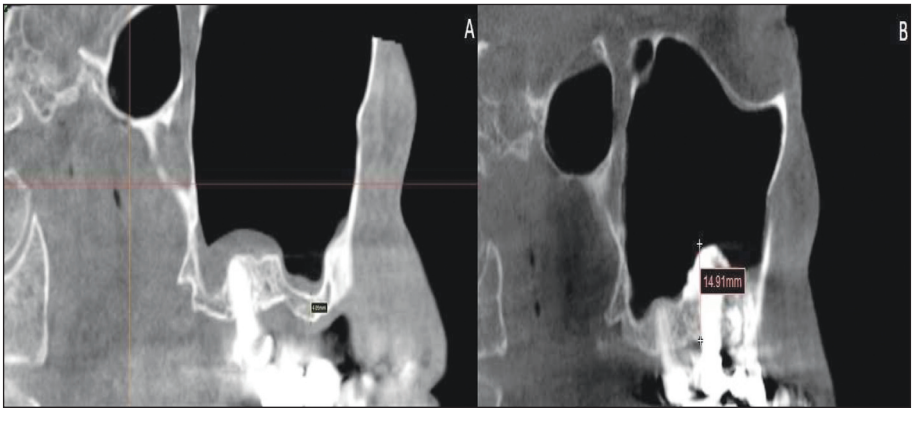

FIGURE 7: A) The measurement of residual ridge height in the midsagittal section. **B)** The measurement of post-operative ridge height in the midsagittal section.

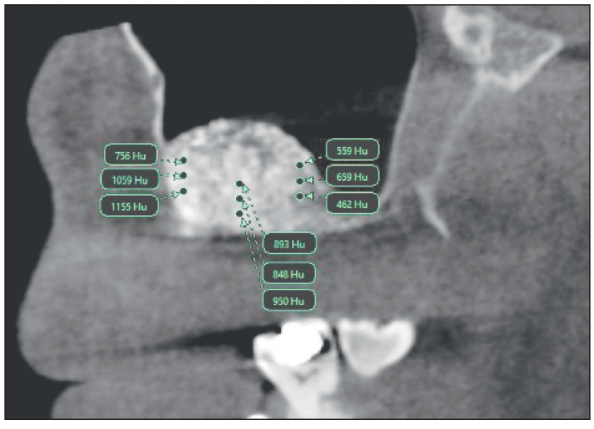

FIGURE 8: Hu measurements: anterior, middle and posterior in the sagittal section, based on anterior, middle and posterior reference points.

ously with sinus floor augmentations. The follow-up period of the patients after augmentation varies between 19 and 25 months (Table 1).

The pre-operative and post-operative volume measurements in Groups 1 and 2 were shown in Table 2. The new bone formation in Group 2 was significantly higher than in Group 1 (Table 2). A moderate positive correlation was observed between residual and post-operative ridge heights in Group 1 ($p<0.05$, $r=0.048$), whereas there was no significant relationship between residual and post-operative ridge heights in Group 2 ($p>0.05$) (Table 3).

The mean HU value was 511±169 for Group 1 and 673±214 for Group 2. These values correspond to Type 2-3 bone considering the classification by Lekholm and Zarb.[12] There was a statistically significant difference in HU values between the 2 groups ($p<0.05$) (Table 4).

DISCUSSION

There is no consensus on the type and origin of the sinus bone graft due to the conflicting reports comparing numerous grafting materials or blood derivatives. Stacchi et al. reported that newly regenerated bone in the sinus floor 12 months after lateral sinus augmentation procedure does not correlate with bone grafting material used in the augmentation procedure.[13] On the contrary, Gultekin et al. reported that bone stability provided by the demineralized bovine bone graft is greater than composite graft that included demineralized bone allograft and biphasic calcium phosphate 6 months after sinus floor augmentation surgery.[14] Klein et al. suggested that bone graft volume is increased with the use of deproteinized inorganic bovine bone 8 months after sinus augmentation surgery.[15] Olgun et al. reported that titanium-prepared PRF used in sinus floor augmentation reached similar levels of new bone formation and maturation with bone allograft in a shorter period.[16] Choukroun et al. used PRF in combination with bone allograft in sinus augmentation and compared the biopsies taken post-operatively from cases augmented with PRF and demineralized freezed dried bone allograft (DFDBA) allograft combination and allograft only.[10] The results showed that DFDBA mixed with PRF clot 4 months after surgery showed similar levels of new bone regeneration compared to DFDBA 8 months after surgery. As consistent with this particular study, the new bone volume in PRF and demineralized cortical-cancellous bone allograft mixture was significantly higher than allograft-only group in the present study.

Bone substitutes are mainly particulate bone grafts that are used alone or as a mixture with autogenous grafts in varying granule sizes and originate from xenogeneic and allogenic sources or manufactured from hydroxyapatite crystals in laboratory conditions. The addition of PRF fragments to the bone substitutes is one of the clinical techniques used to establish a contribution to the therapeutic process in the osteoconduction phase and it is suggested that the addition of PRF in bone grafts increases the bone regeneration potential of particulate bone grafts and prevent bacterial infection.[10,17] It was suggested that the combination of PRF and other bone grafting materials should be done when a delayed implant placement approach is necessary and the bucco-palatal dimension of the sinus was above 10 mm.[18] In a study by Kassolis and Reynolds, platelet-rich plasma (PRP) was used as an adjunct to FDBA in sinus floor augmentation and it was reported that the use of FDBA with a resorbable membrane demonstrated significantly low levels of vital bone formation compared to FDBA and PRP mixture.[19] On the other hand, it was suggested that current evidence supporting the beneficial effect of PRF addition to bone graft in

TABLE 1: New bone volume was calculated by subtracting pre-operative sinus volume and post-operative sinus volume in sinus floor augmentations performed with the use of 2 different graft materials of demineralized corticocancellous bone allograft and the mixture of platelet-rich fibrin and demineralized corticocancellous bone allograft.

Case	Pre-op sinus volume (mm^3)	Post-op sinus volume (mm^3)	New bone volume (mm^3)	Follow-up (months)
Group 1				
1	15309.00	15031.00	278.00	19
2	15340.00	14392.00	948.00	20
3	13346.00	13099.00	247.00	21
4	13078.00	12411.00	667.00	18
5	13011.00	12031.00	980.00	18
6	13486.00	12583.00	903.00	23
7	12118.00	11521.00	597.00	20
8	12840.00	12162.00	678.00	18
9	12948.00	12168.00	780.00	19
10	14842.00	13844.00	638.00	22
11	15340.00	14792.00	548.00	21
12	15217.00	14620.00	597.00	18
13	12340.00	11695.00	645.00	19
14	12560.00	11875.00	685.00	24
15	13992.00	13794.00	198.00	22
16	12220.00	11358.00	862.00	19
17	13905.00	13351.00	554.00	22
18	14430.00	13892.00	538.00	19
19	12147.00	11644.00	503.00	25
20	12023.00	11504.00	519.00	18
Group 2				
1	14056.00	13159.00	897.00	20
2	13562.00	12704.00	858.00	19
3	15341.00	15178.00	163.00	24
4	13540.00	12637.00	903.00	25
5	13454.00	12561.00	893.00	23
6	13670.00	13177.00	493.00	18
7	14210.00	13393.00	817.00	19
8	14113.00	13310.00	803.00	22
9	14340.00	13597.00	743.00	19
10	14356.00	13659.00	697.00	24
11	12980.00	12337.00	643.00	23
12	13142.00	12135.00	1007.00	18
13	13149.00	12293.00	856.00	22
14	13450.00	12639.00	811.00	25
15	14568.00	13647.00	921.00	24
16	14344.00	13565.00	779.00	18
17	13128.00	12031.00	827.00	23
18	12040.00	11139.00	901.00	21
19	13662.00	13013.00	649.00	20
20	12375.00	11743.00	632.00	23
21	13597.00	12634.00	963.00	22
22	12554.00	11887.00	667.00	24
23	14976.00	14429.00	547.00	21

Group 1: Demineralized corticocancellous allograft group; Group 2: Platelet-rich fibrin+demineralized corticocancellous bone allograft group.

TABLE 2: Group 2 demonstrated significantly increased new bone formation compared to Group 1.

	Group 1	Group 2	t value	p value
Mean new bone volume (mm^3) ($\bar{X}$±SD)	618.25±216.12	759.56±186.43	-2.30	0.026*

*Statistically significant; Group 1: Demineralized corticocancellous bone allograft group; Group 2: Demineralized corticocancellous bone allograft and platelet-rich fibrin combination; SD: Standard deviation.

TABLE 3: Relationship between residual ridge height and post-operative ridge height in Group 1 and 2 was analysed by Pearson correlation test.

	Residual ridge height (mm) ($\bar{X}$±SD)	Post-operative ridge height (mm) ($\bar{X}$±SD)	p value	Correlation coefficient
Group 1	5.76±1.34	13.74±1.04	0.048	0.447
Group 2	5.40±1.26	13.73±1.65	0.242	0.254

Group 1: Demineralized corticocancellous bone allograft group; Group 2: Demineralized corticocancellous bone allograft and platelet rich fibrin combination; SD: Standard deviation.

TABLE 4: Statistically significant difference was observed in HU values between two groups.

	$\bar{X}$ (HU)	SD	Minimum (HU)	Maximum (HU)	p value
Group 1	511.69	169.16	255.00	1050.00	<0.05
Group 2	673.07	214.99	354.00	1166.00	

Group 1: Demineralized cortical-cancellous bone allograft group; Group 2: Demineralized cortical-cancellous bone allograft and platelet rich fibrin mixture group; HU: Hounsfield unit; SD: Standard deviation.

sinus augmentation is limited.[20] Nizam et al. reported that the addition of L-PRF to demineralized bovine bone mineral did not improve the new bone regeneration in sinus floor augmentation at 6-month follow-up control.[21] The PRF and demineralized bone allograft combination showed new bone regeneration in the present study. The increase in bone regeneration with the use of PRF may be attributed to several properties of PRF. One of them is the vascularization enhancement effect of PRF and this feature leads to fast new bone formation due to the increased blood flow and neo-angiogenesis in the augmented sinus cavity.[18] As second, the sticky nature of PRF might have had an attribution to the graft integrity by collecting graft particles together preventing the spread, therefore, providing a suitable environment for bone regeneration with osteoconduction.

Kwon et al. reported that structural CBCT analysis can be reliably used in the evaluation of the quality and quantity of sinus bone graft materials.[22] Similar to the current study, Alayan and Ivanovski used a 3-D volumetric analysis program that measures the air-filled sinus cavity with a segmentation tool for the proper establishment of sinus cavity borders to compare the new bone formation between xenograft/autogenous bone mixture and collagen stabilized xenograft in a prospective randomized trial.[23] Volumetric measurement of the changes in the sinus cavity is an improved way of displaying the graft volume excluding the disadvantages of the 3-D thresholding technique and the technique of perimeter tracing of grafting material.[24,25] The marking of graft borders in tomographic sections may be challenging especially in matured sinus bone grafts due to the difficulty of separating the line between the bone graft and native bone. In that sense, the measurement of the perimeters of an air-filled sinus cavity segment by segment in both pre-operative and post-operative conditions may be a more simple and efficient way to properly measure the bone graft volume. Volume reduction of the sinus bone graft, especially after the function of dental implants placed with sinus augmentation, is a reported fact in the following post-

surgical period. The volume change in the bone graft is caused by graft resorption during the bone regeneration period.[15,26] Berberi et al. reported that the volume of mineralized bone cortical allograft deployed in the sinus augmentation procedure is decreased by 20.63% at the end of 1-year function after loading.[27] In the current study, CBCT imaging was performed only in pre-operative and post-operative 18 to 24 months controls to minimize the radiation dose. An immediate post-operative tomographic image is needed to comment on the reduction of sinus graft volume. Moreover, the primary aim was to compare the bone regeneration capacities of demineralized bone allograft and the mixture of demineralized cortical-cancellous bone allograft and PRF. Therefore, no comparison between these 2 graft modalities was made in terms of bone graft shrinkage.

One of the factors determining the success of the sinus lift augmentation procedure is the quality of the newly formed bone, which allows for a high vital bone/implant contact area.[28] The measurement of CBCT data processed with imaging software showed comparable results to bone core biopsies in the evaluation of bone density after sinus floor augmentation.[29] In the present study, HU measurement was made to evaluate the bone quality in the newly regenerated bone areas in two groups and the bone density in the study cases corresponded to type 2-3 bone density according to the classification of Lekholm and Zarb.[12] Similarly, Melisa et al. reported an average bone density of 586±238 HU, a value also corresponding to Type 2-3 bone according to the Lekholm and Zarb classification.[11,12]

Residual ridge height is the height of the alveolar bone before the sinus floor augmentation and new bone formation. The height of the ridge increases with the new bone regeneration in the elevated sinus. Speculation of a possible correlation between residual bone quantity and post-operative new bone quantity may be made due to the expectation of increased new bone formation in higher ridges. It is reported that there is no relationship between residual ridge height and post-operative ridge height after sinus floor augmentation.[23] However, the residual ridge height seems to have a moderate relationship with post-operative ridge height in the bone allograft group, whereas, there is no correlation between residual and postoperative ridge height in demineralized bone allograft and PRF mixture group in the current study. In that situation, it can be speculated that PRF adjunction to bone allograft provides the potential of new bone regeneration in sinus floor augmentation independent of residual ridge height, and the sole usage of allograft alone produces new bone that significantly increases with an increase in residual ridge height. Alayan and Ivanovski compared the residual ridge height and the quantity of sinus graft volume to evaluate the relation between the quantity of residual bone and post-operative new bone formation.[23] In the current study, the residual ridge height was compared to post-operative ridge height to standardize the units. Limitations of the study were listed as;

1. Although there was a difference in regenerated bone volumes between demineralized bone allograft and PRF and demineralized bone allograft mixture group, no comment can be made in regards to bone quality in augmented sinuses. Histomorphometric sampling could not be made due to the retrospective nature of the study.

2. The regenerated bone may undergo dimensional changes in the long term and a follow-up beyond 2 years may have demonstrated less difference between groups.

CONCLUSION

PRF and demineralized cortical-cancellous bone allograft combination may enhance higher levels of new bone formation compared to demineralized bone allograft alone in sinus floor augmentation. HU measurements obtained from a newly regenerated bone in CBCT data may show similar values to that of native bone, showing that the bone quality may be comparable. The mixture of bone allograft and PRF provides new bone regeneration in sinus floor augmentation regardless of the residual ridge height. New ridge height obtained after sinus floor augmentation with demineralized corticocancellous bone allograft may be associated with residual ridge height.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

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