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EVALUATION OF TITANIUM PREPARED PLATELET RICH FIBRIN IN BONE REGENERATION IN LATERAL WINDOW MAXILLARY SINUS AUGMENTATION WITH DELAYED IMPLANT PLACEMENT (RANDOMIZED CONTROLLED CLINICAL TRIAL)

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ABSTRACT

INTRODUCTION: Titanium prepared platelet rich fibrin (T-PRF) has been applied as an alternative to the traditional bone graft in sinus lifting approach.

OBJECTIVES: This trial aimed to evaluate the effect of the use of T-PRF alone on bone formation process as applied in maxillary sinus floor elevation in comparison to traditional T-PRF/bone graft combination as indicated by implant stability as primary objective.

MATERIALS AND METHODS: This clinical trial included 18 patients seeking implant placement in upper posterior maxilla compromised with maxillary sinus pneumatization. All the patients underwent sinus lifting procedure through which a combination of xenograft and T-PRF was used in the control group whereas; T-PRF alone was applied in the test group. Delayed implant placement was applied. Therefore, the time frame of the whole trial is 7 months; 4 months following sinus filling and 3 months after implant placement. The two groups were compared in clinical, radiographic and histological evaluation parameters including bone height, density and volume along with implant stability.

RESULTS: Clinical results revealed that there was no significant difference between T-PRF/ xenograft group and T-PRF group in terms of primary stability (p value=0.811). Radiologically, T-PRF/xenograft showed superior outcomes than T-PRF group in total bone height gain and pre-implant bone density gain (p value=0.001, 0.002 respectively). Histomorphometric results indicated that the difference between the control and test groups was not significant.

CONCLUSIONS: T-PRF alone can be used as an effective grafting material in two stage maxillary sinus augmentation as proven by implant stability as the primary objective measured clinically. Acceleration of bone formation process can be achieved by means of T-PRF whether alone or in combination of xenograft.

KEYWORDS: Sinus lifting, augmentation, platelet concentrate, platelet rich fibrin, PRP.

RUNNING TITLE: titanium prepared platelet rich fibrin for sinus lift.

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INTRODUCTION

Implant placement in edentulous posterior maxilla is considered one of the major dilemmas confronting oral and maxillofacial surgeon. That is mainly related to poor bone quality and quantity in this particular area. A common scenario is represented in maxillary sinus pneumatization following extraction of upper molars yet, compromising residual bone height necessary for implantation procedure. (1)

As a result, several techniques have been developed over the past few decades in order to elevate maxillary sinus floor without perforation of the schneiderian membrane to provoke osteogenic process with or without grafting material. On the other hand, a diversity of biomimetic materials and bone grafts has been investigated to evaluate their healing promotion efficiency and osteogenic ability.Apart from the classical bone grafts, autologous platelet concentrate has attracted more attention in the past 2 decades. Some evidence has been provided regarding its osteogenic, angiogenic and anti-inflammatory impact on both soft and hard tissue regeneration. (1)

Considerable previous studies highlighted the promising act of PRF in bone synthesis whether it is applied as the only biomaterial or in combination with other components as bone grafts or even certain chemicals. (2, 3) In the literature, a debate about application of PRF in maxillary sinus lifting has risen. (4-6) On one hand, a number of scientists

support the principal of applying PRF as a supplemental not as an essential component relying on its modest osteogenic potential.(7) On the other hand, other researches provide evidence regarding effective addition of PRF as a main treatment for bone regeneration either in sinus lifting or other procedures.(7)

As a result of this controversy, it has been emphasized in the literature that preparation of PRF is technique sensitive that implying the importance of standardization of centrifugation protocol and solving the problems observed due to usage of silica coated plastic tubes. Therefore, usage of titanium tubes instead of traditional tubes was suggested. (7, 8-10) As a result, numerous improved approaches and modified versions of PRF have been introduced in order to overcome its shortcomings.

Titanium prepared platelet rich fibrin (T-PRF) was first established by Mustafa Tunalei (10, 11) in 2012 by replacing glass tube or silica coated plastic tube used in the production of classical PRF by titanium tube aiming to overcome the questionable health hazards of silica particles separated during centrifugation of the blood. Tunalei (11) has observed formation of stronger and thicker fibrin network in addition to release of growth factors over a longer period of time than in the typical PRF. Moreover, Tunalei et al. suggested that titanium may trigger platelets more efficiently than silica in other types of tubes. This is in addition to the biocompatibility of titanium that will prevent detrimental health issues reported with casual silica coated plastic tubes.

Importantly, in 2018, a clinical trial of utilization of T-PRF as the only filling material following maxillary sinus floor elevation exhibited promising results as it accelerated bone formation in comparison to allografts. (12) This latter study was accompanied with delayed implants which directed attention towards its possible effectiveness without association with tenting by means of immediate implant. As a result, it could be effective in severe bone resorption cases including residual bone height less than 5 mm in maxillary sinus augmentation. In addition, according to Olgun et al. (12) the application of T-PRF alone in maxillary sinus augmentation has been proven to be clinically and histologically successful. However, another study disputed the improvement of the effect of PRF by centrifugation in titanium tube. (13) Therefore, further well controlled clinical trials are still required to illustrate the exact structure and effect of T-PRF. The Null hypothesis indicates that there is no difference between the outcome of the two groups; a group of T-PRF alone versus another group of conventional T-PRF/xenograft as a filling material in lateral window maxillary sinus lifting procedure. The primary objective is stability of the implant scored by implant stability quotients (ISQ) measured using osstell device *(Osstell ISQ, Göteborg – Sweden)* both immediately after implant placement and at the time of implant uncovering after 3months. Secondary objectives of this trial is to compare the effect of application of T-PRF alone versus conventional T-PRF/xenograft composite as a grafting material in delayed lateral wall maxillary sinus lifting approach as detected by minimum time required for adequate bone formation (healing time), implant survival following placement of prosthesis all over the entire follow up period, parameters of newly formed bone (volume, density, height) as shown in radiological images as well as histological parameters regarding bone surface area ratio and non-mineralized bone surface area ratio.

MATERIALS AND METHODS

2.1 Study design:

This study represents a randomized controlled clinical trial design involving 18 participants requiring sinus lift procedure for the purpose of implant placement. Using coin flipping as a simple randomization method, the patients were randomly and equally divided into two groups each group contains 9 patients; the control group received T-PRF/xenograft as a conventional sub-sinus filling component and the test group underwent maxillary sinus augmentation using T-PRF only.

This study was conducted on patients selected from the outpatient clinics of Oral and Maxillofacial Surgery Department of the Faculty of Dentistry in Alexandria University, Egypt. Written informed consents were obtained from all participants after detailed description of the whole procedures to be performed including its aim. This study was approved from ethics committee and research committee in Faculty of Dentistry in Alexandria, Egypt. Ethical approval number is IRB No. 00010556_IORG 0008839-0301_10/2021. This study followed CONSORT guidelines for conducting clinical trials (figure 1). In addition, it is conformed to the Helsinki declaration of 1975, as revised in 2000 along with the Good Dental Clinical Practice Guide Lines with Alexandria University.

2.2 Participants: Eligibility criteria

Regarding Inclusion criteria, patients were selected by being not less than 18years old, non-smokers, systemically in a healthy condition along with good oral hygiene as indicated by plaque and bleeding scores. In addition, participants should have residual bone height not more than 5 mm in posterior maxilla as measured with CBCT. (12) On the other hand, medically compromised condition such as uncontrolled diabetics, uncontrolled hypertensive patients and congestive heart failure patients were excluded to avoid infections or any adverse reactions related to immunity suppression or any other problems that would compromise bone healing or even surgical procedure. Moreover, patients suffering from acute maxillary sinusitis and those administering any antibiotics or regular anti-inflammatory drugs were also excluded. This was

to prevent the effect of confounding variables and reduce bias. (12)

2.3 Sample size estimation**:**

The minimal sample size was calculated based on a previous study aimed to evaluate the analytical difference between the use of xenograft (control group) and graftless tenting (test group) technique after sinus lift procedure with simultaneous implant placement. Fouad W, et al. (2018) (14) concluded that sinus lift procedures with simultaneous implant placement using xenograft as a filling material or graftless technique are considered reliable procedures, however, the use of xenograft provides better results in all aspects regarding (bone height gain, bone density, and implant stability). Based on their results, adopting a power of 80% to detect a standardized effect size (non-inferiority limit,d) of 10 in stability of the implant scored by ISQ measured using Ostell device (primary outcome), and level of significance 95%(α=0.05), the minimum required sample size was found to be 9 patients per group (number of groups =2) (Total sample size=18 patients). Any withdrawal for any reason was supposed to be compensated by replacement to control for attrition (withdrawal) bias. The sample size was calculated using Gpower version 3.1.9.2.

2.4 Materials:

The conventional therapy used in this trial is the traditional T-PRF mixed with half gram to 1 gram of xenograft *(OneGraft, Corticocancellous Bovine Powder<2mm, German).*T-PRF was the material to be tested in this study as an alternative to classical bone graft. T-PRF was prepared by specific technique established by Tunalei et al. (10, 11) In addition, Sinus lateral window kit was used to perform sinus lift procedure *(Dentis Save Lateral Kit & instruments, Korea)*. Trephine bur *(Trephine drills, China)* 3mm in diameter is utilized to take a bone column sample before implant placement for the purpose of histological analysis.

Moreover, Suitable implant system *(Neodent, Helix GM, Brazilian contents, Germany)* was used in addition to oseodensification kit *(Densah bur, Pakistan)* that was used to promote primary stability for both groups. Implant stability was measured by resonance frequency analysis. Primary stability was measured at the time of implant placement. Secondary Implant stability was measured at the time of uncovering after 3 months.

2.5 Maxillary sinus augmentation (1st surgical intervention)

Lateral window sinus floor lifting procedure was operated under local anesthesia (modified Cald well-Luc sinus augmentation). A paracrestal incision was cut with one or two vertical releasing incisions to create full thickness mucoperiosteal flap.

Round or elliptical bony window was cut 5mm away from the bone crest using piezoelectric device. Careful elevation of the antral mucosal lining was done with delicate manipulation to avoid any perforations. The schneiderian membrane lifting was initiated from the sinus floor toward the posterior wall then superior wall and finally to the anterior wall. For optimal graft placement, elevation of the membrane from the medial wall of the sinus was accomplished as well.

A bellow effect (movement of the sinus membrane with respiratory rhythm) upon patient's breathing was observed for all the patients during the operation indicating the sinus membrane was intact. Following sinus membrane elevation, the sub-sinus cavity was filled with T-PRF/xenograft for control group or T-PRF alone in test group. T-PRF was prepared by specific technique established by Tunalei et al. (11) as follows (figure 2):

20ml blood sample was drawn from the antecubital vein or dorsal metacarpal veins of each participant right or left arm or back of the hand using 20ml syringe. Collected blood was immediately transferred into a 10ml grade IV titanium tube *(Tunalei especially manufactured titanium tubes, Turkey*) without anticoagulant. Each sample was quickly centrifuged using a specific table centrifugation device *(80-1 centrifuge, china)* (878g for 12 min) at room temperature. T-PRF clot was removed from the tube with sterile tweezers then separated from the base of the red blood cells utilizing sterile scissors. The collected T-PRF was divided into 2 parts; one part was divided into minute pieces to be mixed with xenograft in case of control group or to be used solely for test group. The other part was squeezed between 2 glass slabs to form membrane like structure to be used as a barrier against fibrosis between the bone graft and the mucoperiosteal flap. This was followed by replacement of the mucoperiosteal flap and suturing by simple interrupted suture technique utilizing black silk suture.

The patients were instructed to apply cold fomentation and to avoid hot drinks and food for the first 24 hours. From the second day post-operatively, patients were advised to commence hot fomentation for further 24 hours. In addition, patients were directed to avoid eating on the side of the surgery. Oral hygiene recommendations were provided.

Post–operative medications were provided to the patient including (12): Amoxicillin 1gm, clavulanic acid 125mg tablets (Augmentin 1 gm.) combined with metronidazole (500mg tablets) were provided twice daily for 5 to 7 days, Anti-inflammatory analgesics: Diclofenac potassium (Cataflam 50 tablets) was taken three times a day for 7 to 10 days, Anti-edematous drug: chymotrypsin (Alphintern) 2 tablets half an hour prior eating three times a day for 5 days, Chlorhexidine mouth washes 0.12%: three times per day after 24 hours

for 10 day period, Ephedrine nasal drops: 3 to 5 times per day for 5 days.

2.6 Delayed implant placement (2nd surgical intervention)

Delayed implant placement was performed after 4 months for both groups. Crestal incision with reflection of a full mucoperiosteal flap was done under local anesthesia. Small points were marked with the use of marker burs on the area where implants were located. The number of implants for each patient ranged from 1-2 implants according to the prosthetic plan for the patient. However, only one implant was selected to represent the results of each patient. The selection was performed depending on the specific location and its relation to determined bone measures representing the eligibility criteria.

Bone samples were collected from these points by means of 3mm trephine burs to be located 2 to 3mm shorter than the implant length. Preservation of bone specimens were done at room temperature in covered formaldehyde containers (10%). (15) The implant fixture of suitable length and diameter was placed by the typical technique followed by measuring primary implant stability using osstell device and then fixture was sealed with a cover screw.

The flap was repositioned and sutured with black silk suture. Immediate Post-operative instructions and medications were provided to the patients and sutures were removed after one week. After 3 month of implant positioning, CBCT was performed to ensure proper implant placement and bone formation and to compute post-implant bone measurements. Clinically, implants were exposed and healing abutments were installed to prepare for prosthetic phase.

2.7 Clinical assessment:

Clinical assessment of the participants was determined the day following surgery then after one week, two weeks for each surgery and 4 and 7 months post-operatively. The outcomes of the trial were estimated by measuring level of pain using numerical rating scale NRS from 0-10, where 0 reading indicates no pain, (1-3) indicates mild pain, (4-6) shows moderate pain and (7-10) reflects severe pain. (16) Additionally, edema and dehiscence of the wound were detected by inspection of the area to determine if they were present or not. Nasal congestion or bleeding was identified by direct questioning of the patient. Furthermore, Implant stability was measured by resonance frequency analysis. Primary stability was measured at the time of implant placement. Secondary implant stability was measured at the time of uncovering after 3 months.

2.8 Radiological assessment:

Firstly, CBCT was performed preoperatively and after 4 months of sinus lifting and 3 months after implant insertion for all participants. To evaluate post-operative bone gain radiologically, two imaging soft wares *(OsiriX Lite DICOM Viewer, Swiss\ Horos DICOM Viewer GNU Lesser General Public License, Version 3.0, (LGPL 3.0), UK)* were used for analyzing bone volume in centimeter cubic. These soft wares allow determination of bone image in circular slides. The application is then used to collect and compute the bone volume of these slides. In addition, three dimensional radiological softwares *(OnDemand 3D°TM, Korea)* were utilized to measure bone height in millimeters and density in Hounsfield Units. This was done to allow comparison with the preoperative parameters and opposing measures of the other group in addition to determining the location of the implant to be placed along with implants' length and diameter. Three types of bone gain were calculated. Firstly, subtraction of pre-implant bone measurement (height, density or volume) from preoperative bone measurement was done to determine amount of bone gain as a result of sinus lifting only. Secondly, pre-implant bone measurement was subtracted from post-implant bone measures after 3 months to identify amount of bone gain resulted from implant placement solely. Finally, total bone gain of the whole process was computed by subtraction of preoperative bone measures form post-implant bone measures

2.9 Histological assessment:

The bone specimens collected prior to implant placements were fixed directly in 10% neutral buffered formalin, then, rinsed in distilled water, decalcified in 8% hydrochloric acid, dehydrated in ascending grades of alcohol, cleared in xylene and finally embedded in paraffin wax. 5 µm thick serial sections were prepared, cut and stained with haematoxylin and eosin stain to evaluate the newly formed bone (15) as well as Masson-Goldner Trichrome stain using the conventional methods for detection of non-mineralized bone. (17)

2.10 Histomorphometric assessment:

Specimens were inspected by means of Olympus light microscope *(Olympus BX41 Phase Contrast & Darkfield Microscope, Olympus Corporation, Japan).* From each specimen, three serial sections were selected and a digital microscopic camera *(Olympus DP20 digital microscope camera, Olympus Corporation, Japan)* was used to obtain images for these serial sections for both groups where histomorphometric measurements were obtained using Fiji image j software *(National Institutes of Health, USA)* followed by calculation of the mean value. Two parameters were calculated (18):

a) Bone surface area ratio:

Photos of H&E stained slides magnified ×40 were utilized for tracing the bone surface area on the software and the percentage of bone is calculated in relation to the whole surface area of the field.

b) non-mineralized bone ratio:

Images of Masson-Goldner Trichrome stained specimens with × 40 magnifications were adjusted in color deconvolution mode to differentiate shades of the field. Red confined areas were measured to compute the non-mineralized bone surface area ratio in relation to the entire surface area of the field. (17)

2.11 STATISTICAL ANALYSIS

Data were collected, summarized using the IBM SPSS statistics version 29 to be statistically analyzed and compared with the proper statistical analysis methods. Normality of distribution for different variables including age, sex, bone height, density and volume along with histomorphometric variables were investigated with Shapiro-Wilk test. (19, 20) Data were displayed as mean and standard deviations. Paired student t-test was applied to estimate significance levels within each group to compare pre and post-test values. On the other hand, detection of statistical significance between the two groups was determined using both independent samples t-test and One-way ANOVA test. (21)

RESULTS

3.1 Sample characteristics

This trial involved 18 participants requiring sinus lift procedure for the purpose of implant placement. The patients were randomly and equally divided into two groups each group contains 9 patients; control group that received T-PRF/xenograft as a conventional sub-sinus filling component and test group underwent maxillary sinus augmentation using only T-PRF.

A Shapiro-Wilk's test (19,20) ($p>.05$) and a visual inspection of their histograms, normal Q-Q plots and box plots revealed that all variables including age, pre and postoperative bone heights, width, density and volume in addition to histological results were approximately normally distributed for both control and test groups.

There was no significant difference between control and test groups regarding age (table 1) and gender (table 2). This clinical trial contains 18 maxillary sinus augmentation procedures including 35 implant insertions. For each lifting operation, only one implant was selected to represent the bony measurements according to its specific location in relation to the sinus pre and post-operatively. The patients were allocated both equally and blindly between the two groups taking into account that each group consisted of 9 cases; 5 females and 4 males. The mean age of the participants was 41.33±4.743 in the T-PRF/Xenograft control group and 42.89±7.607 in the T-PRF alone test group.

3.2 Clinical results:

The three stages of the trial were performed safely in both groups with no serious complications including sinus membrane perforation, failure of implant primary stability, infection post-operatively. Mild degree of bleeding was observed during surgery in two patients and was controlled successfully with pressure. Normal post-surgical swelling was observed in all the patients. However, it was completely resolved in 4- day- duration. Moderate degree of pain was recorded in all patients following maxillary sinus lifting operation in both groups which was managed effectively by medication and disappeared within 5-7 days. Few drops of blood were reported to get from nostrils following surgery in 3 patients in the first 24 hours post-operatively. Final implant based restorations were fabricated to all the patients in suitable timing.

Primary and secondary implant stability was reported with resonance frequency analysis method. Four different points surrounding each implant was measured to calculate their average to be the representative of implant stability quotient variable ISQ. Within each group, P value was recorded to be 0.971 for control group and 0.861 for test group. As a result, there was no significant difference between primary and secondary stability of implants regarding each group separately. At the same time, Utilization of T-PRF as a supporting material in the control group or as a single filling material for sub-sinus cavity in the test group have successfully resulted in acceptable primary stability in less time frame than usual (4 months instead of 6 months). In addition, for both implant stability values, the mean ISQ is 63.9067 for control group and 62.9811 for test group with no significant difference between the two groups where P value was 0.811 for primary stability and 0.559 for secondary stability (table 3).

3.3 Radiological results

Bone height, density and volume were statistically analyzed within each group (figure 3): and between groups (figure3, figure 4) as following:

3.3.1Bone height:

Regarding bone height, there was significant difference between preoperative bone height and pre-implant bone height after 4 month of first surgery within each group separately (P value < 0.001 for both groups). Whereas there was significant difference between pre-implant bone height and post implant bone height after 3 months for the test group only (P value =0.006 for test group and =0.134 for control group).

To compare between the two groups, it was found that there was significant difference between control and test group regarding the amount of gain in pre-implant bone height (P value=0.001). The height of the bone was increased in higher percentage in control group in comparison to test group after 4 months. At the same time, there was no significant difference in the amount of bone height gain after implant insertion by 3 months (p value=0.216). According to total bone height gain, it was found that there is significant difference

between the two groups in favor of control group (table 4).

3.3.2 Bone density:

Regarding bone density, there was significant difference between preoperative bone density and pre-implant bone density within control group only (P value = 0.003 for control group and = 0.868 for test group). However, there was significant difference between pre-implant bone density and post-implant bone density within both control and test groups (P value = 0.004, 0.001 respectively).

To compare between the two groups, it was found that there was significant difference between control and test group regarding the amount of gain in pre-implant bone density (P value=0.002). The density of the bone was increased in higher percentage in control group in comparison to test group after 4 months. At the same time, there was no significant difference in the amount of bone density gain in both post-implant insertion by 3 months only (p value=0.220) and total bone density gain for the whole procedure (p value=0.147) (table 5).

3.3.3. Bone volume:

In terms of bone volume, there is significant difference between preoperative bone volume and pre-implant bone volume for test group only (P value= 0.008 for test group and = 0.193 for control group). Conversely, there was significant difference between pre-implant bone volume and post-implant bone volume within control group solely (P value=0.002 for control group and= 0.286 for test group).

To compare between the two groups, it was found that there was no significant difference between control and test group regarding the amount of gain in all three stages; pre-implant bone volume gain, post-implant bone volume alone gain and total bone volume gain (P value=0.904, 0.445 and 0.506 respectively) (table 6).

3.4 Histological results:

In both control and test groups, histological examination revealed formation of numerous bone trabeculae surrounding bone marrow spaces. However, thicker bone trabeculae were observed in control group in comparison to test group (figure 5 a, d). Whereas upon higher magnification, newly formed bone was observed including osteocytes and numerous resting and reversal lines (figure 5 b, c, e, and f).

In both groups, Goldener Masson Trichrome stain showed homogenously mineralized bone trabeculae (stained green) with minute scattered areas of unmineralized bone (stained red) (figure 6 a, b, c and d).

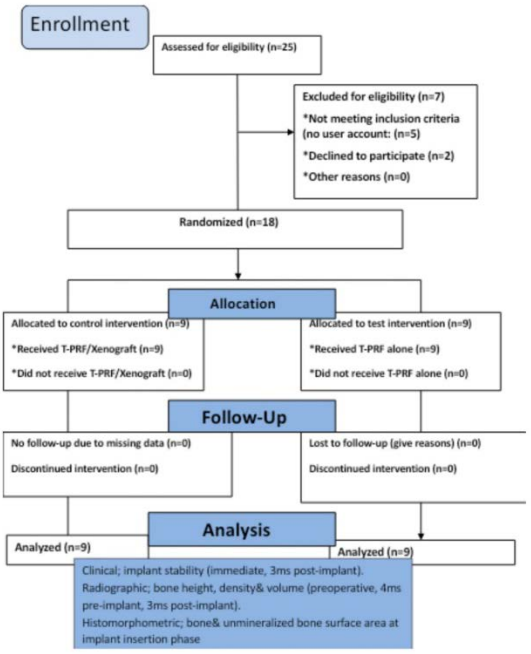

Figure 1: Consort 2010 flow diagram.

Figure 2 a) 20ml blood sample was drawn from the antecubital vein or dorsal metacarpal veins of each participant right or left arm or back of the hand using 20ml syringe and was immediately transferred into a 10ml grade IV titanium tube without anticoagulant. b) The collected T-PRF was divided into 2 parts. c) One part of T-PRF was inserted in the sub-sinus cavity in test group.

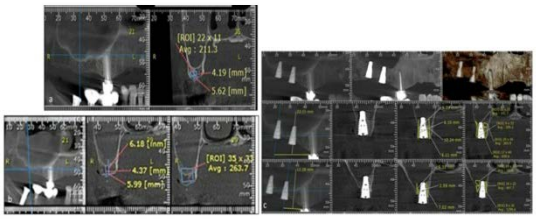

Figure 3 Cone beam computerized tomography CBCT for case 1(test group) showing a) preoperative bone measurements (bone height, width and density). b) Pre-implant radiograph CBCT after 4 month of sinus lift showing bone height and density. c) CBCT after implant placement by 3 month showing bone height, density.

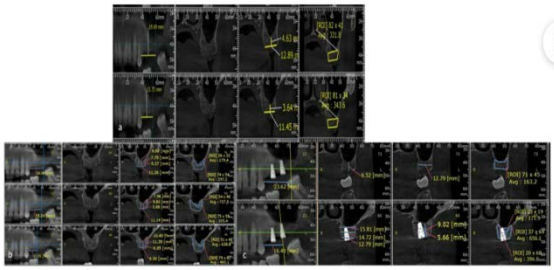

Figure 4 Cone beam computerized tomography CBCT for case 2(control group) showing a) preoperative bone measurements (bone height, width and density). b) Pre-implant radiograph CBCT after 4 month of sinus lift showing bone height and density. c) CBCT after implant placement by 3 month showing bone height, density.

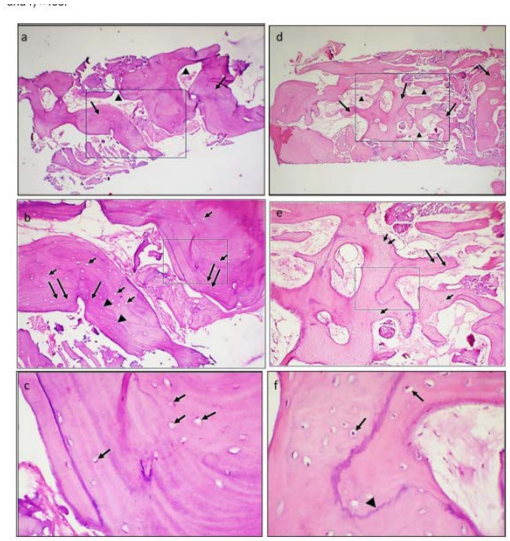

Figure 5. Light micrograph (LM) of bone samples of control group (a, b, and c) and test group (d, e, and f). Control group a) bone sample consists of thick bone trabeculae (arrows) surrounding bone marrow tissues (arrow heads). b) A higher magnification of previous micrograph inset showing the structure of the newly formed bone which contains osteocytes (short arrows), numerous resting lines (long arrows) and reversal lines (arrow heads). c) A higher magnification of previous micrograph inset showing the regularly distributed osteocytes' lacunae (arrows). Test group d) bone sample consists of numerous cancellous bone trabeculae (arrows) surrounding bone marrow tissues (arrow heads). e) A higher magnification of the previous micrograph inset showing the structure of the newly formed bone which contains irregularly distributed osteocytes (short arrows) in some areas and resting lines (long arrows). f) A higher magnification of previous micrograph inset showing osteocytes' lacunae (arrows) and reversal line (arrow heads). H&E (a and d) ×40, (b and e) ×100, (c and f) ×400.

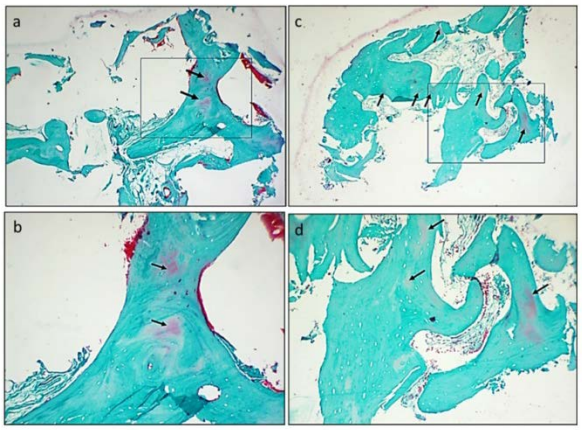

Figure 6 LM of bone samples of control group (a and b) and test group (c and d) showing areas of homogenously mineralized bone trabeculae (stained green) and unmineralized bone (stained red) (b and d) are higher magnification of (a and c) respectively. Goldener masson trichrome stain (a and c)× 40, (b and d) ×100.

3.5 Histomorphometric results:

Statistical analysis revealed that there was no significant difference in bone surface area percentage between control and test group (P value = 0.884). In addition, the difference of non-mineralized bone surface area ratio between T-PRF/Xenograft control group and T-PRF test group is not significant as well. (P value=0.625) (table 7).

Table 1 statistical description of patient age.

	Group		Test of significance P value
	T-PRF/xenograft control group n*=9	T-PRF test group n=9	
Age (years)			
Mean±SD†	41.33±4.743	42.89±7.607	P‡= 0.610 NS§
Min. - Max.¶	35-50	32-55	

*: Number of patients. †: Standard deviation. ‡: Probability of error (chance). §: Statistically not significant (p≥0.05). ¶: Minimum to maximum. Tests of significance: to compare between the two groups are independent T-Test and One way Anova.

Table 2 statistical description of patient gender

Patient group	**T-PRF/Xenograft (control group) n*= 9**		**T-PRF n=9**	
Patient gender	Female	Male	Female	Male
Patient number	5	4	5	4

***: Number of patients**

Table 2 T-PRF/Xenograft vs. T-PRF in implant stability.

Implant stability (ISQ)	Group		Test of significance P value
	T-PRF/xeno graft control group	Sole T-PRF test group	P*=0.811 NS†
Primary stability ISQb			
Mean± SD‡	63.9067± 7.96526	62.9811±**8.19 001**	
Min.-Max.§	54.50-75.29	48.00-71.50	
Secondary stability(after 3months) ISQc			P=0.559 NS
Mean± SD	64.0133± 5.25541	62.5589±5.07 044	
Min.-Max.	55.00-69.25	56.75-72.25	
Paired sample significance (within each group) p value			
Primary stability/Secondary stability ISQb/ISQc	P=0.971 NS	P=0.861 NS	

*: Probability of error (chance). †: Statistically not significant (p≥0.05). ‡: Standard deviation. §: Minimum-Maximum. Tests of significance: to compare between the two groups are independent T-Test and One way Anova.

DISCUSSION

Platelet-Rich Fibrin has been found to have superior quality than other grafting alternatives due to its high content of platelets which are fundamental component in the healing process of both soft and hard tissues. (22) An innovative modification of PRF namely T-PRF have dragged attention due to its higher qualities than traditional PRF. (11, 12)

In the present study, maxillary sinus lift with delayed implant was done due to severe bony deficiency with residual bone height ranging from 3.64 to 4.97mm in both groups along with low bone density vary from 28 to 410.22 HU in control group and from 42.70 to 560.50 HU in test group. This was most likely expected to compromise immediate implant fixation and reliable primary stability. In the present clinical trial, application of T-PRF alone as a filling material for sub-sinus cavity with residual bone height (less than 5mm) with delayed implant placement was evaluated clinically, radiologically and histologically in comparison to conventional T-PRF/Xenograft.

In this clinical trial, it has been shown that T-PRF alone has the ability to provide the same results produced by mixture of T-PRF and xenograft when used to fill the sub-sinus cavity with delayed implant fixation relying on clinical and histological analysis. These are approximately the same results of Olgun trial (13), in 2018, which compared the results of using of T-PRF alone versus allograft in maxillary sinus lifting after 4 month and 6 month respectively. The present study goes in line with Olgun trial in presenting beneficial effect of T-PRF. That is, time required for bone regeneration has been reduced from 6 to 4 month by application of T-PRF alone or even by addition of T-PRF to Xenograft with more rapid implant placement as shown in both groups.

According to the outcomes of both groups in the present study, T-PRF, as an advanced generation of PRF, succeeded to form bone in less time in comparison to other grafting materials. This comes in agreement with two studies (23, 24) reported that addition of PRF to freeze dried bone allograft reduced the time required for bone regeneration from 8 to 4 months in addition to a trial conducted in 2019 (25) suggested that use of L- PRF in addition to deproteinized bovine bone mineral accelerated and enhanced bone formation process in maxillary sinus lifting approach.

Depending on the results obtained by the present study, addition of T-PRF to corticocancellous bovine bone xenograft hastened regeneration of bone sufficient to provide suitable primary stability for implants placed earlier than conventional timing. This comes in contrary to a systematic review presented by Ali, S. and his colleges (26), in 2015, which concluded from 5 studies (23, 24, 27-29) that addition of PRF to demineralized freeze-dried bone allograft DFDBA has accelerated bone regeneration process and reduced the healing time. However, it has no significant influence when applied with deproteinized bovine bone xenograft. The contradicting results could be explained by the superior quality of T-PRF as compared to traditional PRF.

In terms of implant stability measured clinically, the results of the present study revealed comparable implant stability in both groups. This goes in line with conclusion of a systematic review done by Inchingolo et al. (30) in which inclusion of PRP in bone graft used in sinus lift procedure has been shown to promote primary stability of the implants in comparison to using bone graft alone taking into consideration that T-PRF in the present study was included in both groups.

However, in the present study, it has been found that T-PRF/xenograft group showed higher pre-implant radiographic bone height gain and total bone height gain compared to T-PRF group. These results are in agreement with the results of kempraj et al. (31), in 2020, which re-evaluated Choukroun's PRF as the only filling material for the sub-sinus cavity in delayed implant protocol of lateral window technique as compared to Xenograft used alone. They reported significant difference between control and study group in the radiologically measured bone height in the favor of the Xenograft group. (31) These results could be

attributed to the resilience of fibrin matrix of PRF impeding its ability to perform the role of well-structured scaffold against the schneiderian membrane which compress the plug of PRF. (32) At the same time, in the current trial, it was observed that the difference between pre-implant bone height and post-implant bone height was significant only in the test group which could be explained by the tent pegs' effect of inserted implant and its ability to compensate the less pre-implant bone height in the test group and enhance the prognosis of the test group.

Despite the fact that bone height gain before implantation was higher in control group than test group, the primary stability of both groups was not significantly different. This entails that the amount of bone formed by T-PRF alone was sufficient to achieve the main goal of the whole process which is obtaining a suitable primary stability to allow success of implantation procedure as shown in the present study.

Regarding bone density, in the present study, it has been found that T-PRF/xenograft group showed higher radiographic pre-implant bone density gain compared to T-PRF group. These results go in line with the results of kempraj et al. (31), in 2020, which reported significant difference between control and study group in the radiologically measured bone density in the favor of the Xenograft group as well which are most likely due to the less resilience of xenograft scaffold in comparison to fibrin mesh in general. (32) At the same time, in the present clinical trial, total bone density gain was reported to be not significantly different between the two groups which in the contrary to Kempraj et al. results. This could be explained in favor of superior quality of T-PRF compared to the Choukroun's PRF used by kempraj et al. (31)

In the current trial, although the increased value of radiographic bone density gain before implant placement was significantly different between both groups in favor of control group, this did not negatively influence the primary stability of the implants. Additionally, it has been remarkably compensated by the implant insertion to the extent made there is no significant difference between both groups in post-implant bone density gain and total bone density gain. By another meaning, any advantage presented by the traditional T-PRF/Xenograft could be compensated for the test group after implant placement taking into consideration the achievement of the primary implant stability in the first place for both groups similarly.

In terms of bone volume, it has been reported in the present clinical trial that there is no significant difference between radiographic pre-implant, post-implant and total bone volume gain in both groups. This is in the contrary to the outcomes obtained from Olgun et al. clinical trial (13) which revealed greater percentage of radiographic bone volume gain in favor of the allograft control group. This difference in the results could be explained by using T-PRF as a supporting material to xenograft in the control group of the present study which provides comparable results in both groups. At the same time, it was reported that allograft is capable of accelerating bone remodeling in comparison to traditional xenograft. (33) This indicates the importance of applying other trial that compare T-PRF alone and T-PRF/ allograft composite.

The present study emphasized, histologically, the ability of T-PRF to enhance both bone deposition and healing process whether used alone or in combination with other bone graft with no significant difference between both groups. This goes in line with a recent systematic review (30) conducted on 22 studies aiming to analyze the results of most recent studies for better identification of the capabilities of the autologous platelet concentrates displayed as platelet-rich plasma PRP, platelet-rich fibrin PRF and concentrated growth factors CGF. In that systematic review (30), it has been mentioned that there is histological evidence regarding the ability of growth factors to promote blood supply in addition to acceleration of new bone generation. In addition, introduction of PRF into the bone graft have been proven to enhance bone deposition depending upon natural healing process of human body due to the angiogenic capacity of PRF that provokes healing process in the surgical area. This is highly noticeable in surgical wounds with deficient blood supply including sinus floor elevation.

In the present study, histomorphometric analysis has shown that mean of bone surface area ratio was 36.42 in control group and 35.43 in the test group which are not significantly different. Moreover, average value of non-mineralized bone appeared by trichrome stain was detected to be 8.41 and 8.1 in control and test group respectively which showed no statistical significant difference between the two groups as well. At the same time, Systematic review of Inchingolo et al. (30) for 5 work papers (34-38) conversely concluded that newly formed bone ratio measured histologically is greater in the case of application of CGF alone, another derivative of PRF, as compared to Xenograft in the delayed sinus-lift procedure. (37) This could be justified by stiffer fibrin matrix of CGF than PRP, PRF. (39) These contradicting results between the current study and the previous studies (30) could be clarified by using T-PRF in both groups in the present study that led to similar histomorphometric results between both groups that did not applied in case of CGF study which was used only in one group. (37)

On the other hand, in the current trial, histological indications of new bone formation detected by

multiple resting and reversal lines along with less ratio of non-mineralized bone than mineralized bone in both groups support the potential of T-PRF to reduce required bone formation period from 6 to 4 months group which allow earlier implant placement than in the conventional techniques. Another point that was observed from histological analysis is that despite of the difference in graft volume clinically between both groups which was higher in the control group; this did not impede the maturation process of newly formed bone. However, thicker bone trabeculae were observed in the control group which could be attributed to remnants of resorped bone graft.

These histological outcomes of the present study along with other results in the literature emphasize the role of the T-PRF in guidance healing of hard tissues. These results come in agreement with the histological outcomes stated by Choukroun et al. (23), in 2006.

In the present study, although radiological analysis revealed better bone height and density in case of applying T-PRF/Xenograft than in T-PRF alone. This is actually expected as xenograft is appearing radiopaque in x-rays. In addition, microscopic results are more significant in relation to bone regeneration. Therefore, both clinical and histological outcomes have been taking mainly into consideration. However, the radiological results are implying that T-PRF doesn't completely fulfill the scaffold structure requirements needed for typical bone deposition necessary for implant fixation. But the clinical and histological results are still satisfactory and emphasize the favorable role that could be obtained from application of T-PRF by its ability to replace classic grafting material or by reduction of healing time to 4 month.

To summarize, this study introduces more evidence to support the claim of reliable effect of T-PRF whether it has been used alone or in combination with other bone graft which has been predicted by Simonpieri (40) via emerging superior modified generation of PRF. Eventually, the successful results in both groups, in the present study, promote two significant hypotheses; firstly, the capability of T-PRF to be used alone in maxillary sinus augmentation even in delayed implant protocol and secondly, the reinforcing effect of T-PRF to xenograft and its ability to reduce normal healing time.

Drawbacks of this study involve small sample size, absence of split mouth technique to overcome differential effects. Finally, it has to be mentioned that gender dimensions in this research could not be addressed and is considered as a limitation of this trial s' generalizability. Other well controlled clinical trials with larger sample size and re-evaluation of T-PRF with introduction of suitable scaffold are recommended.

CONCLUSION

This clinical trial supports the effectiveness of T-PRF in bone formation capacity as advanced generation of traditional PRF which was used in every day practice as a supplemental not as a main grafting material in maxillary sinus augmentation particularly with delayed implant placement.

Finally, T-PRF alone is effective grafting material in two stage maxillary sinus augmentation as indicated by implant stability as the primary objective measured clinically. This has been supported by histmorhometric measurements as well. In addition, acceleration of bone formation process can be achieved by means of T-PRF to reduce healing time from 6 to 4 months.

Ethical approval: This study was approved from ethics committee and research committee in Faculty of Dentistry in Alexandria, Egypt. Ethical approval number is IRB No. 00010556_IORG 0008839-0301_10/2021.

Patient consent: Written informed consents were obtained from all participants after detailed description of the whole procedures to be performed including its aim.

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