Comparing the effect of Leukocyte PRF (L-PRF) and Advanced PRF(A-PRF) added to nanohydroxyapatite on osseous regeneration after maxillary cyst enucleation (Randomized controlled trial)

Original Article

Areeg A. Elsakka [1], Moustafa Taha [1], Yasser Nabil [2], Mohamed Diaa [1]

Department of oral and maxillofacial surgery, faculty of dentistry, Ain Shams university, Cairo [1] , Department of oral and maxillofacial surgery, military teaching specialized dental hospital, Military Medical Complex, Kobri El-Kobba., Cairo [2]

ABSTRACT

Purpose: To assess the difference between the effect of Leukocyte PRF (L-PRF) and Advanced PRF(A-PRF) added to nano hydroxyapatite bone graft on osseous regeneration after cyst enucleation.

Methodology: This clinical study was a Randomized Controlled Trial on 21 patients seeking treatment of maxillary cyst and randomly allocated into 3 groups each group containing 7 patients. In group A, a blood sample was collected for preparation of A-PRF and added to nano hydroxyapatite bone graft to fill the bony defect after cyst enucleation. In group B, L-PRF was prepared and added to nanobone to fill the defect. While group C was a negative control group with no grafting of residual defect.

Results: Regarding CT preoperative and postoperative volumetric measurements of the defect, there was a significant difference between different groups (p=0.002). Defect volume was reduced by 80.72% in group A followed by group B which reduced by 62.36% then group C reduced by 21.49 %.Also, there was increase in bone density by 78.56% in group A followed by group B increased by 53.70% while in group C increased by 21.49%.And also, there was 78.64% reduction in size of the residual defect in group A followed by group B reduced by 66.20% and the least group was group C reduced by 48.60%.

Conclusion: A-PRF/nanobone mixture accelerated bone healing and improved both quality and quantity of regenerated bone in residual defects compared to L-PRF/ Nanobone mixture and control groups. And outcomes of L-PRF/ Nanobone mixture were better than control group.

Key Words: Radicular cyst, PRF, L-PRF, A-PRF, Nanobone

Received: 21 March 2024 , **Accepted:** 20 April 2024 .

Corresponding Author: Areeg A. Elsakka , MSc Student at Oral and maxillofacial department,faculty of dentistry,ains hams university,cairo,egypt , **Mobile:** 01010093383 , **E-mail:** areegelsakka@gmail.com

ISSN: 2090-097X, April 2024, Vol. 15, No. 2

INTRODUCTION

Cysts of the jaws are pseudoneoplastic bone lesions characterized by the presence of a cavity lined by an epithelial membrane of odontogenic origin and less often respiratory and filled with a liquid or semiliquid content. [1] Odontogenic cysts are one of the most common pathological lesions of jaw, and they are causing bone destruction, pain especially when are infected, swelling and loosening of teeth. [2]

Radicular cyst is one of the most common odontogenic cysts affecting maxilla three times more than mandible. They arise from epithelial residues in the periodontal ligament because of inflammation, usually following death of dental pulp. The process of pathogenesis of a cyst begins by initiation which gradually progresses to cyst formation and then enlarges to involve the adjacent bone and other vital structures in its surrounding. The toxins from necrotic pulp present here exit at the apex of the tooth, leading to periapical inflammation. [3]

Maxillofacial reconstruction of bony defects is a routine procedure for rehabilitation of patients with severe bone deformities to restore continuity, shape and strength of the jaw. [4] A variety of treatment modalities including the use of autogenous bone grafts and bone substitutes materials, guided tissue regeneration (GTR) with the use of barrier membranes, and growth factors have been proposed to promote bone regeneration. [5]

Bone grafts and bone regenerative materials are used for treating intrabony defects in periapical surgery with varying degrees of success. Autograft is associated with high degree of donor site morbidity and allograft is associated with risk of disease transmission, which pushed the clinicians toward searching for more different autologous material, such as platelet-rich plasma (PRP) and platelet-rich fibrin (PRF), and production of a large number of alternative bone substitute acting as osteoconductive or osteoinductive materials , to avoid the risk of tissue morbidity and disease transmission. [6,7]

 DOI: 10.21608/OMX.2024.278499.1233

One of these osteoinductive materials is nanocrystalline hydroxyapatite which displays enhances biomechanical properties that more closely mimics the composition of natural bone.[8] The nanostructure allows for a larger surface to volume ratio, promoting more effective adhesion, proliferation, and differentiation of osteogenic progenitor cells, so nHA could encourage bone formation by local osteoblasts at sites of alveolar bone reconstruction. [9,10]

On the other hand, the use of autologous platelet concentrates for bone healing in oral and maxillofacial region was first introduced by Robert Marx in 1998. [11] Platelet-rich fibrin (PRF) described by Choukroun et al. in 2001 is a second-generation platelet concentrate after PRP contains platelets and growth factors in the form of fibrin membranes prepared from the patient's own blood free of any anticoagulant or other artificial biochemical modifications as bovine thrombin which known as inhibitors of wound healing. [6,12,13]

PRF contains many biologically active growth factors that stimulate tissue repair mechanisms, such as chemotaxis, cell proliferation, angiogenesis, extracellular matrix deposition, and remodeling, and cytokines, that stimulate bone and soft tissue healing. So PRF is considered an easy and cost-effective way for both soft and hard tissue regeneration. [14,15] Since high centrifugation forces are known to shift cell populations to the bottom of collection tubes (whereas PRF is collected from the top one-third layer), so it was recently hypothesized that by reducing centrifugation speed (G-force), will increase the leukocyte numbers within the PRF matrix. The solid form that resulted from this low-speed concept, called advanced platelets-rich fibrin (A-PRF), was presented in 2014 by Choukroun et al. [16,17] Furthermore, it was found that the release of several types of growth factors, as PDGF, TGF-β1,VEGF, epidermal growth factor (EGF), and insulin-like growth factor (IGF), in advanced PRF(A-PRF) were significantly higher than in leukocyte PRF (L-PRF) and PRP.[18]

This study was aimed to assess the difference between the effect of Leukocyte PRF (L-PRF) and Advanced PRF(A-PRF) added to nano hydroxyapatite bone graft substitute on osseous regeneration after radicular cyst enucleation.

MATERIAL AND METHODS

2.1 Patients selection

This study was conducted on 21 medically free patients whose ages ranged from 20 to 50 years, seeking treatment of maxillary cyst and reconstruction of residual moderate size bone defect after cystic enucleation. Exclusion criteria were presence of acute or chronic infection at the site of grafting that may affect bone and soft tissue healing.

All patients were treated at Department of oral and maxillofacial surgery, faculty of dentistry, Ain Shams university, Cairo, Egypt and Department of oral and maxillofacial surgery, military teaching specialized dental hospital, Military Medical Complex, Kobri El-Kobba, Cairo, Egypt.

2.2 Pre-operative phase

C.T scan and digital panoramic radiograph was done to determine the site and dimensions of the defect. Oral hygiene instructions and oral hygiene measures. Chlorhexidinegluc-onate (0.12%) * mouthwash was prescribed for all patients three times daily for seven days pre-operatively, Antibiotic**(amoxicillin and clavuric acid) and analgesics***.

2.2 Operative phase

Preparation of PRF

10 ml of peripheral venous blood was collected by butterfly needle with tube holder in 2 plain glass tubes without anticoagulant from cephalic vein and immediately placed with a third glass tube filled with saline for balance in LC-04R centrifuge and centrifuged at 3000 rpm for 10 minutes for (L-PRF) and at 1500 rpm for 14 minutes for (A-PRF) (figure 1).

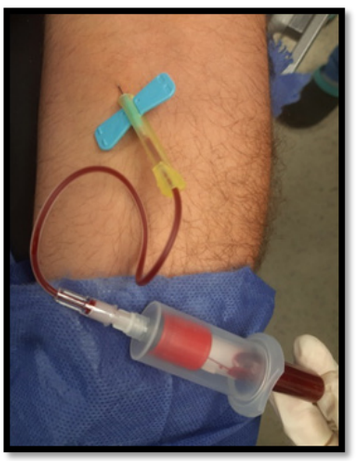

Figure 1: collection of blood sample

A fibrin clot is then obtained in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma at top. Platelets are trapped massively in the fibrin meshes (figure 2). The upper straw coloured layer is then removed and middle fraction is collected, 2 mm below lower dividing line, which is the PRF (figure 3,4)

Figure 2: 3 layers in the glass tube

* Augmentin™, GlaxoSmithKline, Egypt.
** Brufen®, Abbott, Egypt.
***Alphintern®, Amoun, Egypt.

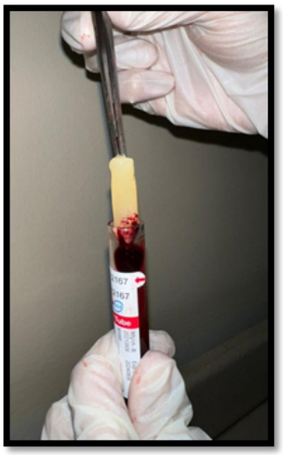

Figure 3: PRF clot

Figure 4: PRF clots in PRF box.

Surgical procedure

Iodine solution used to carry out extraoral antisepsis. Following administration of local anaesthesia, mucoperiosteal flap was designed and according to the extent of the lesion and reflected (figure 5). Enucleation of the lesion using bone curettes (figure 6,7). This was followed by proper debridement of the defect site and irrigation with sterile saline solution. The cystic lesions were sent for histopathological examination (figure 8).

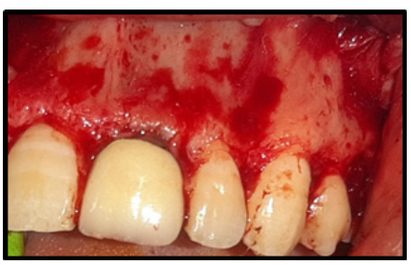

Figure 5: mucoperosteal flap reflected.

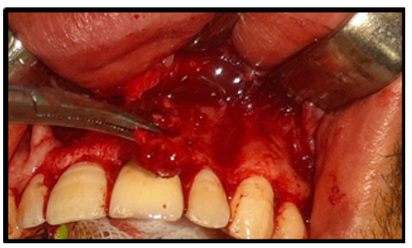

Figure 6: Enucleation of the lesion.

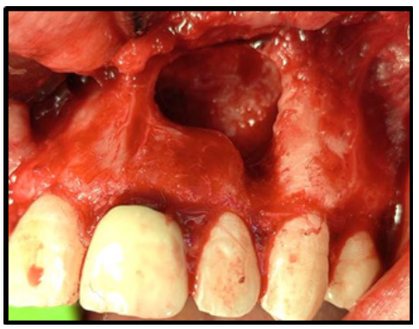

Figure 7: lesion was removed.

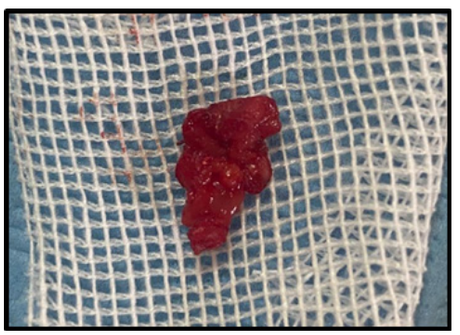

Figure 8: Cystic lesion.

All patients were randomly allocated into 3 groups by simple random sampling method, each group containing 7 patients. Group A (study group) was grafted with A-PRF mixed with Nanobone* in residual surgical defects (figure 9), Group B (study group) was grafted with L-PRF mixed with Nanobone in residual surgical defects. Then application of PRF membrane as a barrier (figure 10). While Group C (control group) is negative control group with no grafting for the residual surgical defects. Then mucoperiosteal flap was repositioned and secured in place using 3-0 resorbable surgical suture (figure 11).

* Artoss GmbH, 18069 Rostock, Germany.

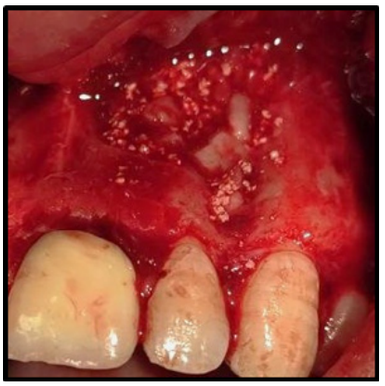

Figure 9: Application of PRF mixed with nanobone

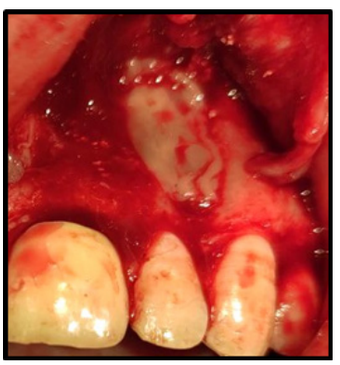

Figure 10: PRF membrane coverage

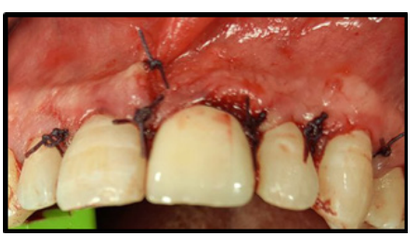

Figure 11: Flap repositioning and closure

Postoperative follow-up

Antibiotics were prescribed to all patients in the form of 1 gm of Amoxicillin and Clavulanate potassium* BID for five days post-operatively, non-steroidal anti-inflammatory drug in the form of Ibuprofen 400 mg** TID for after meals for four days, anti-edematous*** TID for three days and 0.2% chlorhexidine gluconate solution as a mouth rinse for a period of five days. Routine postoperative instructions were given to the patients and sutures were removed after 7 days. Computed tomography was performed on the 6th month post-operatively for the assessment of healing of the bony defects regarding both defect volume and size and bone density using Planmeca Romexis software.

1-Measuring the change in defect volume.

By using DICOM files which were treated with Planmeca Romexis Viewer 5.2.1.R multiplanar reconstruction view (coronal, axial & sagittal) (figure 12-17).

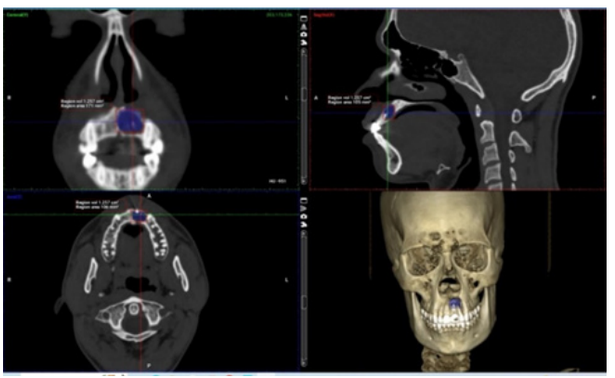

Figure 12: Preoperative volumetric measurement in group A (A-PRF).

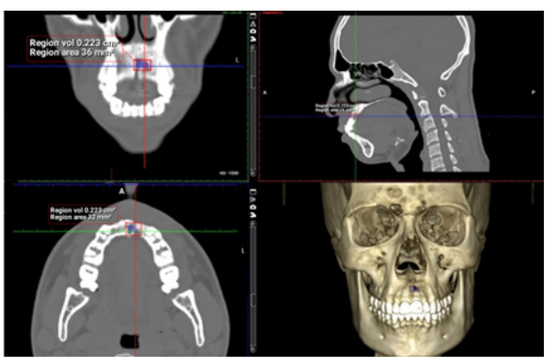

Figure 13: Volumetric measurement after 6 months in group A (A-PRF).

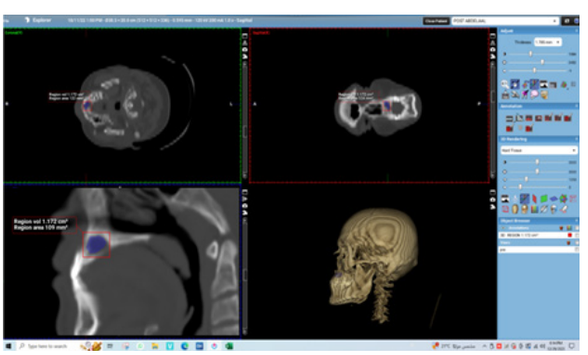

Figure 14: Preoperative volumetric measurement in group B(L-PRF).

* Augmentin™, GlaxoSmithKline, Egypt.
** Brufen®, Abbott, Egypt.
***Alphintern®, Amoun, Egypt.

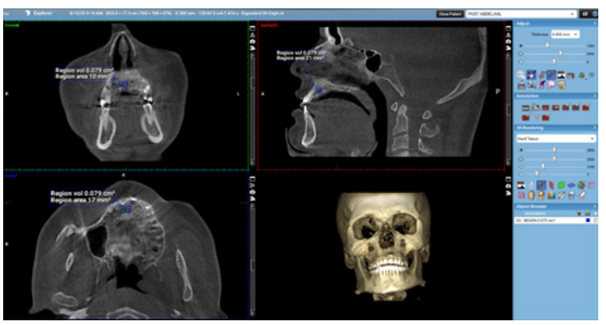

Figure 15: Volumetric measurement after 6 months in group B

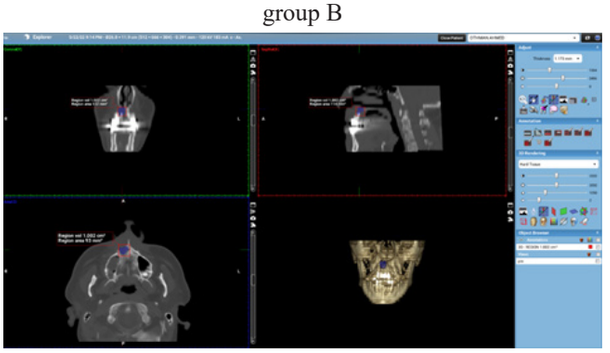

Figure 16: Preoperative volumetric measurement in group C(control).

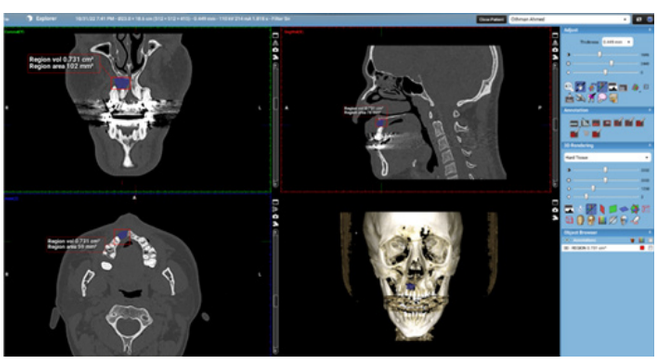

Figure 17: Volumetric measurement after 6 months in group C (control).

2- Measuring the improvement in pixel intensity inside ***the defect that refers to the estimated density.***

Regarding the bone density, it was calculated using the Hounsfield Unit through the ROI (Region of Interest) within the software. By selecting a tool of draw square from Annotation Box then 15×15 mm square was adapted at the midline every 3mm from the hard palate as a reference point through the lesion for three times and the density average was calculated (figure 18-23).

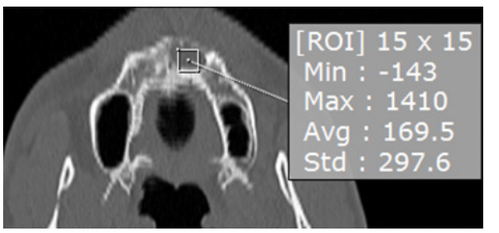

Figure 18: Preoperative bone density measurement in group A (A-PRF).

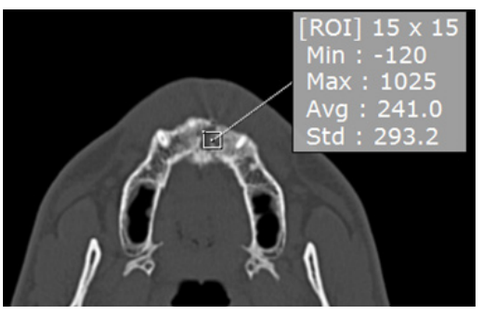

Figure19: Preoperative bone density measurement in group A (A-PRF).

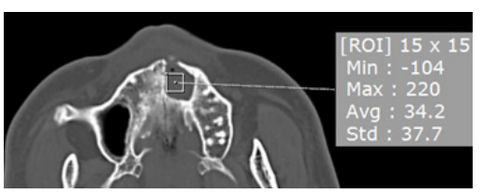

Figure 20: Preoperative bone density measurement in group B (L-PRF).

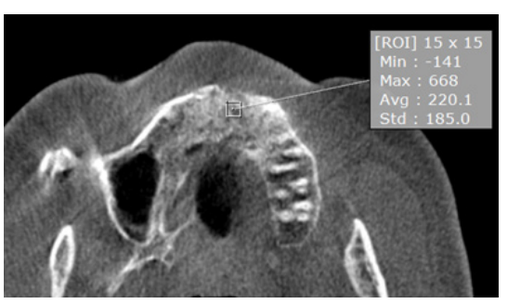

Figure 21: Postoperative bone density measurement in group B (L-PRF).

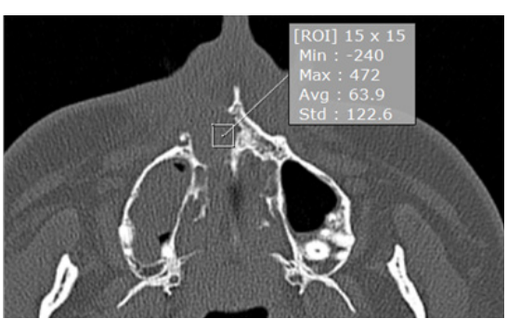

Figure 22: Preoperative bone density measurement in group C (control).

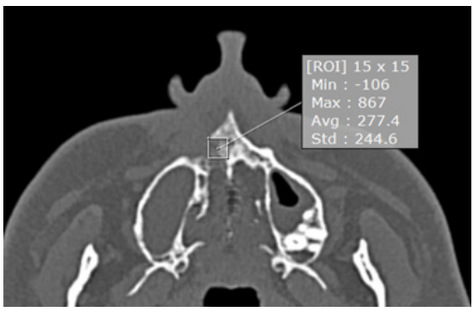

Figure 23: Postoperative bone density measurement in group C (control).

3- Measuring the change in defect size.

Surface area was measured preoperatively and 6 months postoperatively for all patients and the change in size was analyzed (figure 24-29).

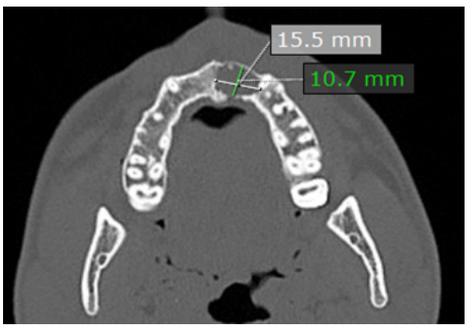

Figure 24: C.T of maxillary cyst showing preoperative size of the lesion in group A (A-PRF).

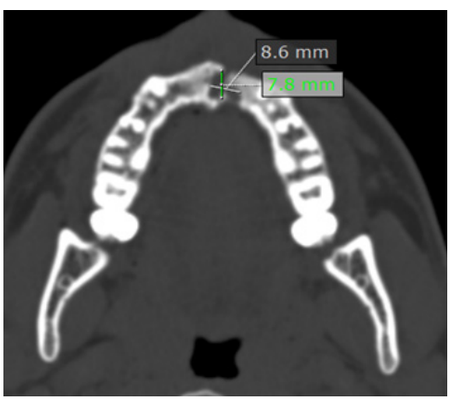

Figure 25: Postoperative size of the residual defect in group A (A-PRF).

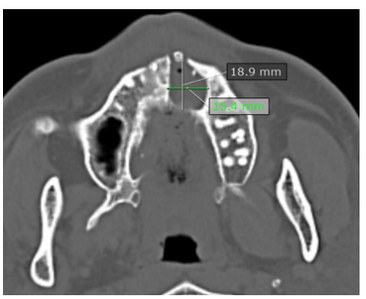

Figure 26: C.T of maxillary cyst showing preoperative size of the lesion in group B (L-PRF).

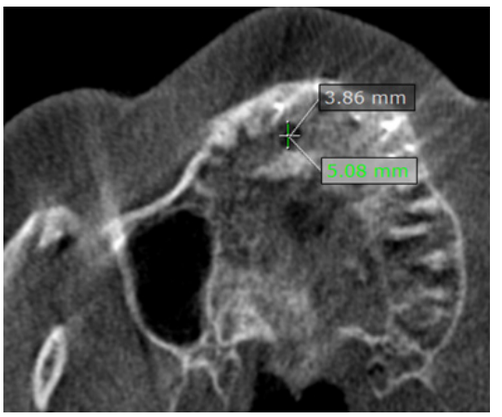

Figure 27: Postoperative size of the residual defect in group B (L-PRF).

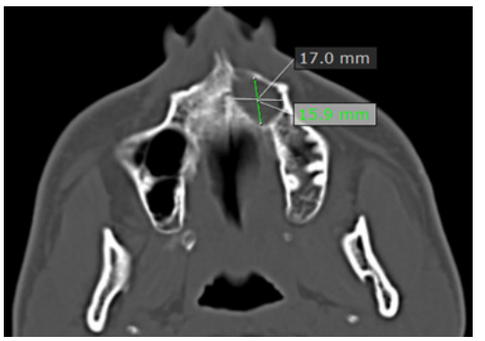

Figure 28: C.T of maxillary cyst showing preoperative size of the lesion in group C (control).

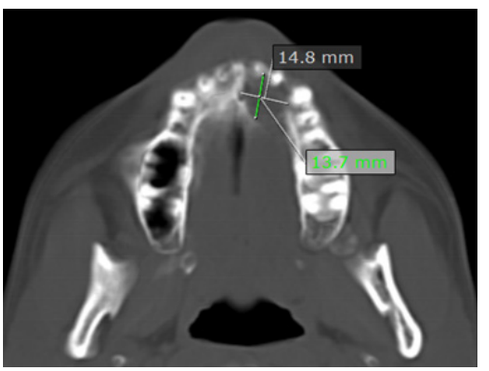

Figure 29: Postoperative size of the residual defect in group C (control).

RESULTS

Numerical data were presented as mean and standard deviation values. They were analyzed for normality using Shapiro-Wilk test. Data were non-parametric and were analyzed using Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni correction. The significance level was set at $p<0.05$ within all tests.

Statistical analysis was performed with R statistical analysis software version 4.3.1 for Windows .

3.1 Analysis of volumetric changes

Regarding the preoperative and 6-month postoperative volumetric measurements of the defect, there was a significant difference between different groups (P=0.002). We found that defect volume was reduced by 80.72% with mean/SD of 1.33±0.31 mm3 in group A (A-PRF / Nanobone) followed by group B (L-PRF / Nanobone) which reduced by 62.36% with value of 0.82±0.12 mm3 then group C (control) reduced by 21.49 % with value of 0.44±0.12 mm3. Which means that the largest amount of bone formation was in (group A) followed by (group B) then (group C) (figure 30).

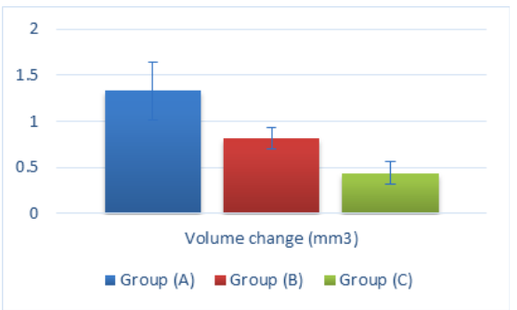

Figure 30: Bar chart showing mean and standard deviation values of volumetric change (mm3) for different groups.

3.2 Analysis of density changes

There was a significant difference between different groups (P=0.002).

Bone density was increase by 78.56% with mean/SD value of 248.64±40.48 HU in A-PRF group followed by L-PRF group increased by 53.70% with value of 120.06±32.95 HU while in control group increased by 21.49% with value of 34.03±10.03 HU (figure 31)

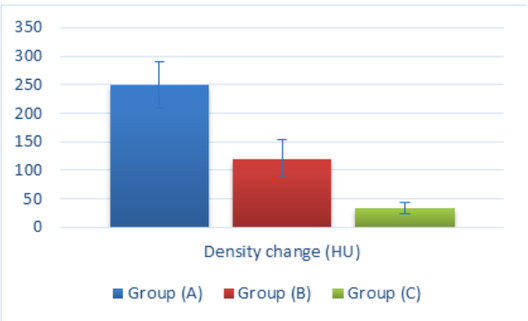

Figure 31: Bar chart showing mean and standard deviation values of density change (HU) for different groups.

R Core Team (2023). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

3.3 Analysis of size change of the defect.

There was a significant difference between different groups (P=0.004). There was 78.64% reduction in size of the residual defect with mean/SD value of 131.20±30.30 mm2 in A-PRF group followed by L-PRF group which reduced by 66.20% with value of 103.36±18.53 mm2 and the least group was the control group which reduced by 48.60% with value of 70.21±8.66 mm2 (figure 32).

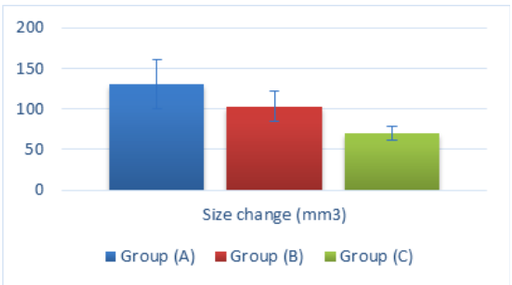

Figure 32: Bar chart showing mean and standard deviation values of size change (mm2) for different groups.

DISCUSSION

Regeneration of bone after periapical surgery depends primarily on wound closure, angiogenesis of vessels, source of undifferentiated mesenchymal cells, space maintenance and stability of the wound. [19] Many research advocates grafting critical-size bone defects following enucleation of odontogenic cysts or tumors to accelerate bone healing and improve the quality and quantity of the regenerated bone. [20,21]

The most commonly used technique for regeneration involves the use of osseous grafts which aids in tissue or bone regeneration through a variety of mechanisms. HA has shown very good results with respect to periodontal and periapical bone regeneration. Literature review reported that a combination of HA and PRF resulted in greater soft tissue healing and better defect fill than PRF used alone. [22] Thus, HA was selected to enhance the effects of PRF by maintaining the space for tissue regeneration and osteoconductive effects in the bony defect area. Bone grafts alone without a blood clotting factor are unlikely to promote periapical wound healing. [23] Biologically, blood clot is a better space filler than all bone grafting materials. A blood clot is the host's own biologic product which plays a major role in wound healing. [24]

Platelet-rich fibrin is prepared naturally without addition of thrombin, and it is hypothesized that PRF has a natural fibrin framework and can protect growth factors from proteolysis. Thus, growth factors can keep their activity for a relatively longer period and stimulate bone regeneration effectively. [25]

Regarding cells quantified histologically within the PRF matrix, Ghanaati et al. was found that the majority of leukocytes were found near the bottom of the fibrin clot in standard L-PRF. Based on this finding, it became clear that centrifugation speeds (G-forces) were evidently too high, pushing leukocytes to the bottom of centrifugation tubes and away from the PRF matrix clot. To redistribute leukocyte cell numbers across the entire PRF matrix, lower centrifugation speeds were investigated. It was confirmed that a higher cell number and growth factors could be obtained, and more platelets were found in the distal part of A-PRF. The increased cell distribution of neutrophilic granulocytes of the A-PRF membrane might indeed be the basis for a better functionality of the transplanted monocytes/macrophages and lymphocytes, and their deployment to support tissue regeneration. [17] Fujioka et al. in 2016 found that PRF can release growth factors gradually and keep their activity to a relatively long period compared with PRP. Although he found that A-PRF released the highest total amount of growth factors when compared to either PRF or PRP over time. [25] Therefore, in the present study, we compared the effect of A-PRF and L-PRF mixed with nanohydroxyapatite on bone regeneration after enucleation of maxillary radicular cysts. The initial diagnosis and treatment plan were performed using CT scan. The radiographic findings were compared to the clinical picture and surgical biopsy report which established the histopathological diagnosis of cystic lesion. The follow up was performed for the assessment of the treatment outcome following cyst enucleation and grafting of the bone defect using CT scan.

Regarding the preoperative and postoperative volumetric measurements of the defect, there was a significant difference between different groups (p=0.002). We found that defect volume was reduced by 80.72% in group A (A-PRF / Nanobone) followed by group B (L-PRF / Nanobone) which reduced by 62.36% then group C (control) reduced by 21.49 %. Which means that the largest amount of bone formation was in (group A) followed by (group B) then (group C). Also, we found that there was an increase in bone density by 78.56% in A-PRF group followed by L-PRF group by 53.70% while in control group increased by 21.49%. And also, there was 78.64% reduction in size of the residual defect in A-PRF group followed by L-PRF group which reduced by 66.20% and the least group was the control group which reduced by 48.60%.

The result of our study showed that the A-PRF/Nanobone mixture accelerated bone healing and improved bone quality and quantity of regenerated bone more than the L-PRF/Nanobone mixture and control group. And L-PRF/Nanobone group is better than control group.

In a study using PRF mixed with Nanobone in bone defects after cyst enucleation, they found that all cases demonstrated accelerated wound healing without any signs of post-operative complications.

There was a 31% reduction of the surface area of the bone defects on the 6th month and 51% size reduction on the 9th month post-operatively. Regarding the bone density, there was an increase of 22.2% in the 6th month, reaching 50.8% by the 9th month. The results of this study showed that the Nanobone/ PRF mixture accelerated bone healing and improved the quality and quantity of the regenerated bone. The rate of increase of the bone density and decrease in the surface area of the bone defects is significantly superior to other studies where the defects were left to heal spontaneously without bone graft. [26]

The same results were obtained by Nacopaulos et al. in 2014 who evaluated the effect of L-PRF in combination with synthetic materials for bone regeneration in rabbits. They showed higher cortical and subcortical bone formation when PRF was combined with synthetic materials than PRF alone and Elgendy et al. in 2015 who showed that nHA in combination with L-PRF, had clinical advantages and increased bone gain and density over the use of nHA alone and [27,28]

In intra bony defect therapy, PRF was found to be beneficial for bone formation. In two clinical studies using PRF in IBD after cystic enucleation conducted by Meshram et. al. and Dar et. al., during follow-up all patients showed obvious and gradual radiographic osseous regeneration. Radiographically, complete bone regeneration and good bone density was seen in all patients within six post operative months. [29,30]

Masahiro et al. in 2019 examined the effects of A-PRF on osteoblastic activity in the socket after tooth extraction in dogs. He found that A-PRF enhances the osteoblastic activity of alveolar bone combined with histological analysis in the experimental group and A-PRF was more rapid than a self-limiting process during induction of bone formation by enhancing osteoblastic activity. [31] Liangjing Xin et al. in 2020 found a significant SM repair occurred when utilizing A-PRF, and the degradation of A-PRF was matched with the SM repair process at an early stage; bone remodeling in the sinus cavity was active, and a greater amount of new bone formation occurred under the perforated SM area in the A-PRF group at a later time point. [32] In 2020 GUPTA et al. found that higher score of bone density was formed in A-PRF group than control group after application in impacted mandibular third molar sockets as we found in our study. [33]
Also, in 2021 lavagen N et al. compared the effect of A-PRF and PRF on bone regeneration in alveolar cleft and he found that higher levels of bone volume were formed in A-PRF group than L-PRF group. [34)] Similar to our study Jamalpour et al. in 2022 found that A-PRF and L-PRF improve bone density more than control group after their application in management of MORNJ, but unlike to our result no statistical difference between A-PRF and L-PRF. [35]

Shah et al. (2023) concluded that A-PRF can be recommended for improved delivery of growth factors and osteogenesis as he found that A-PRF has superior mechanical properties, increased growth factor releases of TGF-b, PDGF-BB, and VEGF as well as superior cell viability, alkaline phosphatase production, and mineralization on human periodontal ligament cells compared to L-PRF and I-PRF. [36]

Although Titirinli et al. in 2017 found that there were no marked differences between A-PRF and L-PRF in regard to the quantity of bone formation and bone quality after application of it in mandibular bone defects in rabbits. He concluded that PRF and its variations have positive effects on the new bone tissue and cell number and may lead to more rapid ossification compared to the unprocessed bone defects but there is no difference between the variations. [37]

In contrast to this study, Da silva et al. in 2022 found no difference between A-PRF and L-PRF in bone volume and new formed bone area when he compared the effect of them on healing of critical size defects in rat calvaria histologically. But also, he found that both groups had significantly higher bone volume and newly formed bone area than those of the control group and higher bone density than control group as we found. [38]

CONCLUSION:

On the basis of the results obtained from this study, it can be concluded that the combined use of A-PRF with Nanohydroxyapatite for bone regeneration following the enucleation of maxillary radicular cysts induced accelerated bone healing and improved both quality and quantity of regenerated bone in residual defects compared to L-PRF/ Nanobone mixture and control groups.

And outcomes of L-PRF/ Nanobone mixture group were better than control group.

CONFLICT OF INTEREST

This clinical study was self-funded by the authors, with no conflict of interest.

REFERENCES:

(1) Sapp JP, Eversole LR, Wysocki GP. Cyst of the oral regions. In: Rudolph P, ed. Contemporary Oral and Maxillofacial Pathology. St Louis, MO: Mosby Inc. an Elsevier Imprint; 2004:46–47

(2) Koca H, Esin A, Aycan K. Outcome of dentigerous cysts treated with marsupialization. J Clin Pediatr Dent 2009; 34(2):165–168.

(3) Schwartz Z, Somers A, Mellonig TJ, Carnes DL, Dean DD, Cochran DL, et al. Ability of commercial demineralized freeze-dried bone allograft to induce new bone formation is dependent on donor age but not gender. J Periodontol. 1998; 69:470-8.

(4) Bernstein S, Cooke J, Fotek P, Wang HL. Vertical bone augmentation: where are we now? Implant Dent. 2006 Sep; 15:219-28.

(5) Chitsazi MT, Shirmohammadi A, Faramarzie M, Pourabbas R, An Rostamzadeh. A clinical comparison of nanocrystalline hydroxyapatite (ostim) and autogenous bone graft in the treatment of periodontal intrabony defects. Med Oral Patol Oral Cir Bucal 2011;16(3): e448e53.

(6) Toffler M, Toscano N, Holtzclaw D, Corso MD, Ehrenfest DD. JIACD continuing education introducing Choukroun's platelet rich fibrin (PRF) to the reconstructive surgery milieu. J Implant AdvClin Dent. 2009;1(6):21–32.

(7) Habibovic P, de Groot K. Osteoinductive biomaterials properties and relevance in bone repair. J Tissue Eng Regen Med 2007;1(1):25e32.

(8) Funda, G.; Taschieri, S.; Bruno, G.A.; Grecchi, E.; Paolo, S.; Girolamo, D.; Del Fabbro, M. Nanotechnology Scaffolds for Alveolar Bone Regeneration. Materials 2020, 13, 201.

(9) Mygind, T.; Stiehler, M.; Baatrup, A.; Li, H.; Zou, X.; Flyvbjerg, A.; Kassem, M.; Bünger, C. Mesenchymal stem cell ingrowth and differentiation on coralline hydroxyapatite scaffolds. Biomaterials 2007, 28, 1036–1047.

(10) Sánchez-Salcedo, S.; Arcos, D.; Vallet-Regí, M. Upgrading Calcium Phosphate Scaffolds for Tissue Engineering Applications. Key Eng. Mater. 2008, 377, 19–42.

(11) Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma- Growth factor enhancement for bone grafts. Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontology 1998; 85:638–46.

(12) Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, et al. (2006) Plateletrich fibrin (PRF): a second-generation platelet concentrate, part I: technological concepts and evolution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 101: e37-e44.

(13) Gupta V, Bains BK, Singh GP, Mathur A, Bains R (2011) Regenerative potential of platelet rich fibrin in dentistry: Literature review. Asian J Oral Health Allied Sci 1: 22-28.

(14) Choukroun J, Adda F, Schoeffler C, Vervelle A. An opportunity in perioimplantol- ogy: the PRF. Implantodontie. 2001; 42:55–62(in French).

(15) Singh S, Singh A, Singh S, Singh R. Application of PRF in surgical management of periapical lesions. Natl J Maxillofac Surg. 2013;4:94–99.

(16) Ghanaati S, Booms P, Orlowska A, et al. Advanced platelet-rich fibrin: A new concept for cell-based tissue engineering by means of inflammatory cells. J Oral Implantol 2014;40:679-689.

(17) Choukroun J. Advanced PRF, & i-PRF: platelet concentrates or blood concentrates. J Periodontal Med Clin Pract. 2014;1:3.

(18) Kobayashi E, Flu¨ckiger L, Fujioka-Kobayashi M, et al. Comparative release of growth factors from PRP, PRF, and advanced-PRF [published online ahead of print January 25, 2016]. Clin Oral Investig. doi:10.1007/s00784-016-1719-1.

(19) Boyapati L, Wang HL. The role of stress in periodontal disease and wound healing. Periodontol 2000, 2007; 44:195-210.

(20) Pradel W, Eckelt U, Lauer G. Bone regeneration after enucleation of mandibular cysts: comparing autogenous grafts from tissue-engineered bone and iliac bone. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101(3):285e90.

(21) Bernab_e PF, Melo LG, Cintra LT, Gomes-Filho JE, Dezan Jr E, Nagata MJ. Bone healing in critical-size defects treated with either bone graft, membrane, or a combination of both materials: a histological and histometric study in rat tibiae. Clin Oral Implants Res 2012;23(3):384e8.

(22) Pradeep AR, Bajaj P, Rao NS, Agarwal E, Naik SB. Platelet-rich fibrin combined with a porous hydroxyapatite graft for the treatment of three-wall intrabony defects in chronic periodontitis: A randomized controlled clinical trial. J Periodontol 2012; 83:1499-507.

(23) Laurell L, Gottlow J. Guided tissue regeneration update. Int Dent J 1998; 48:386-98.

(24) Sunitha Raja V, Munirathnam Naidu E. Platelet-rich fibrin: Evolution of a second-generation platelet concentrate. Indian J Dent Res 2008; 19:42-6.

(25)Kobayashi E, Flückiger L, Fujioka-Kobayashi M, Sawada K, Sculean A, Schaller B, Miron RJ. Comparative release of growth factors from PRP, PRF, and advanced-PRF. Clin Oral Investig. 2016; 20(9):2353-2360.

(26) Eldibany RM, Shokry MM. The effect of Nanobone® in combination with platelet rich fibrin on bone regeneration following enucleation of large mandibular cysts. Tanta Dental Journal, 2014; 11(2), 100–108.

(27) Nacopoulos C, Dontas I, Lelovas P, Galanos A, Vesalas AM, Raptou P, et al. Enhancement of bone regeneration with the combination of platelet-rich fibrin and synthetic graft. J Craniofac Surg. 2014; 25(6):21642168.

(28) Elgendy EA, Abo Shady TE. Clinical and radiographic evaluation of nanocrystalline hydroxyapatite with or without platelet-rich fibrin membrane in the treatment of periodontal intrabony defects. J Indian Soc Periodontol. 2015; 19(1):61–65.

(29) Meshram VS, Lambade PN, Meshram PV, Kadu A, Tiwari MS. The autologous platelet rich fibrin: A novel approach in osseous regeneration after cystic enucleation: A pilot study. Indian Journal of Dental Research, 2015; 26(6), 560.

(30) Dar M, Hakim T, Shah A, Najar L, Yaqoob G and Lanker F. Use of autologous platelet-rich fibrin in osseous regeneration after cystic enucleation: A clinical study. Journal of oral biology and craniofacial research, 2016; 6: S29-S32.

(31) To M, Su C, Hidaka K, Okudera T, Matsuo M. Effect of advanced platelet-rich fibrin on accelerating alveolar bone formation in dogs: a histological and immunofluorescence evaluation. Anatomical Science International, 2019; 94(3), 238–244

(32) Xin L, Yuan S, Mu Z, Li D, Song J, Chen T. Histological and Histomorphometric Evaluation of Applying a Bioactive Advanced Platelet-Rich Fibrin to a Perforated Schneiderian Membrane in a Maxillary Sinus Elevation Model. Frontiers in Bioengineering and Biotechnology, 2020; 8:600032.

(33) Gupta N and Agarwal S. Advanced–PRF: Clinical evaluation in impacted mandibular third molar sockets. Journal of Stomatology, Oral and Maxillofacial Surgery, 2021; 122(1), 43-49.

(34) Lavagen N, Nokovitch L, Algrin A, Dakpe S, Testelin S, Devauchelle B, Gbaguidi C. Efficiency of advanced-PRF usage in the treatment of alveolar cleft with iliac bone graft: A retrospective study. Journal of Cranio-Maxillofacial Surgery, 2021; 49(10), 923-928.

(35) Jamalpour MR, Shahabi S, Baghestani M, Shokri A, Jamshidi S, Khazaei S. Complementarity of surgical therapy, photobiomodulation, A-PRF and L-PRF for management of medication-related osteonecrosis of the jaw (MRONJ): an animal study. BMC Oral Health. 2022; 22(1):241.

(36)Shah R, MGT, Thomas R, ABTK. Advanced platelet rich fibrin demonstrates improved osteogenic induction potential in human periodontal ligament cells, growth factor production and mechanical properties as compared to leukocyte and platelet fibrin and injectable platelet rich fibrin. Oral Maxillofac Surg. 2023; 1-2.

(37) Titirinli K, Tekin U, Atıl F, Önder ME, Şenguven B, Ozgul O, Kocyigit ID. Evaluation of advanced platelet rich fibrin (A-PRF) on bone healing. Is it better than old version? A histological animal study. Journal of Biomaterials and Tissue Engineering, 2017; 7(6), 478-483.

(38) Da Silva LMP, Sávio DDSF, de Ávila FC, Vicente RM, Reis GGD, Denardi RJ, Messora MR. Comparison of the effects of platelet concentrates produced by high and low-speed centrifugation protocols on the healing of critical-size defects in rat calvaria: A microtomographic and histomorphometric study. Platelets, 2022; 33(8), 1175-1184.