

The Use of Platelet Gel with Bone Grafting for Reconstruction of Upper Jaw Defects

OSAMA A. ADLY, M.D.; AMAL S. AHMAD, M.D. and KHALED A. GAD, M.D.

The Departments of General Surgery, Clinical Pathology and Radiology, Faculty of Medicine, Suez Canal University

ABSTRACT

Purpose: In this study, we reconstructed upper jaw defects using bone grafts harvested from iliac cancellous bone incorporation with platelet-rich plasma (PRP) and evaluated its efficacy in osteoregeneration.

Materials and Methods: 15 patients with upper jaw defects underwent iliac bone grafting with PRP. Quantitative evaluation of regenerated bone was made with dental CT scans and compared with controls.

Results: The average of the volume ratio of regenerated bone of reconstructed upper jaw defects in cases with PRP was higher than in controls. There were no complications from the blood draw or PRP.

Conclusion: PRP was a safe and cost-effective source for growth factors and was easy to extract. It could enhance the osteogenesis of alveolar bone grafting in patients with different upper jaw defects and may be useful for subsequent orthodontic therapy.

INTRODUCTION

Autologous iliac bone marrow is used preferably because of its sufficient quantity and high osteoinductive potential. However, even with iliac bone, insufficient osteoregeneration may occur due to several factors such as the patient's age, defect size, influence of functional stress, and others [1-3].

The healing of fractures and bone grafts is accomplished by the interaction of osteoblasts and the extracellular matrix under the influence of various growth factors, of which some are secreted by platelets. These factors can activate the proliferation and differentiation of local osteoprogenitor cells into bone forming cells leading to the formation of bone matrix and mineralization [4-6].

Various attempts have been made to improve bone healing by accelerating the rate of new bone formation and the maturation of the matrix. Bone morphogenetic proteins (BMPs) which obtained

from the blood are known to be strong osteoinductive growth factors and their successful applications in defect and non-union models in small and large animals have been well documented by various groups worldwide [4-7].

In 1998 Marx et al., reported that adding platelet-rich plasma (PRP) or platelet gel (PG) to an autogenous cancellous bone graft caused a faster maturation rate and greater amount of new bone formation in an alveolar defect. The authors claim that when activated with thrombin and calcium chloride the applied platelets in the PRP delivered a high concentration of growth factors into the recipient bed [8].

Although the growth factors and mechanisms involved are still poorly understood, the easy application of PG in clinical practice and its possible beneficial outcome, including bone regeneration, reduction of bleeding and rapid tissue healing, hold promise [9].

In platelet gel, the platelets must be activated to release the contents of their α granules, with the clot that forms providing a vehicle to contain the secreted proteins and maintain their presence at the site of application. The significance behind its use refers to the abundance of growth factors (GF) present in a well prepared PRP concentrate such platelet-derived growth factor (PDGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), insulin like growth factor (IGF), platelet-derived angiogenic factor, and epithelial growth factor (EGF) [10]. When analyzed by enzyme-linked immunosorbent assay, a 7-fold increase in TGF, 30-fold increase in PDGF, and 10-fold increase in EGF were seen in PRP compared with whole blood [11]. It has been known that PDGF may affect wound healing including bone as it promotes revascularization at the trauma site, collagen synthesis, and mitogenesis of stem

cells and osteoblasts [12]. TGF- β participates in the inhibition of osteoclastic activity by stimulating chemotactic migration of osteoblasts to the site of injury [13].

Although mechanical stabilization has been a hallmark of orthopaedic surgical management, orthobiologics are now playing an increasing role. Recent studies have shown that combining PG with bone or bone substitutes present significant faster radiographic maturation and denser bone regeneration histologically. Surgical sites enhanced with PG have been shown to heal at rates two to three times that of normal surgical site [14]. Thus, PG can be a great adjunct to many surgical procedures such as bone grafts, and maxillofacial reconstructions. It is recently been hypothesized that due to the co-linearity between growth factors and bone regeneration, PG and auto bone chips could have an important function in this process, while they are full of fresh concentrated platelet.

As autologous platelet gel offer an easy and cost-effective way to obtain high concentrations of growth factors for tissue healing and regeneration, the objective of this study was to evaluate the effect of platelet gel and autogenous iliac crest graft on bone regeneration in different upper jaw defects.

MATERIAL AND METHODS

This is a randomized controlled clinical trial conducted in The Plastic Surgery Unit, SCUH from June 1998 to June 2010. It involved 30 reconstructed patients with various upper jaw defects. They include 12 patients with tumour resection, 16 patients with secondary alveolar cleft repair, and 2 patients with segmental post-traumatic bone loss.

Any patient with severe illness (as CRF, CHF...), history of hematological disorders, mental or psychological disorders, and prior to chemotherapy or radiotherapy was excluded from the study. Preoperatively; all patients were subjected to full medical and dental history, routine lab investigations, diagnostic radiographs and photography.

A- Graft preparation: Skin incision 5-6cm long will be made along the lateral edges of posterior iliac crest in case of using graft from posterior iliac crest. The incisions will be along the anterior edges of anterior iliac crest in case of using graft from anterior iliac crest. An autogenous cortico-cancellous bone block depending on the site of the defect will be harvested and additional cancellous bone will be harvested if needed. The cancellous bone will be milled and

stored on isotonic water. The iliac periosteum will be tightly sutured, followed by closure of remaining wound in 2 layers.

B- Preparation of PRP: A closed system of ordinary blood packs was used throughout the process. We used standard triple packs (Baxter KGR 7340) containing citrate phosphate dextrose (CPD) as an anticoagulant for autologous (or homologous) blood collection procedure that was done at least 48h before the PG application.

Platelets separation: The PRP was separated from the blood by centrifugation for 15min at 327g and at ambient temperature (soft spine). Recentrifugation at 3000g for 15min (hard spine) was done to concentrate platelets. The platelet concentration target was preset at $>6 \times 10^{10}$ /unit and a plasma volume of 40-60ml, then kept for 48h at +20°C with continuous shaking on a horizontal shaker (Forma Scientific, Marietta, OH) [13,14].

Thrombin preparation: Platelet poor plasma was mixed with calcium gluconate (1/0.2 rate) into sterile Falcon tube and then incubated at +37°C for 15-30min; the final supernatant, full of thrombin precursors, is recovered and kept into syringe at -40°C. It was important to remove the thrombin solution from the clot as soon as possible, as thrombin adsorbs to it [15].

Platelet gel activation: From the platelet concentrate pack, a volume withdrawn into syringe. Thrombin syringe, thawed at +37°C. Added in sterile Falcon tube with calcium gluconate in the following proportions: 3 parts of platelet concentrate, 1 part of thrombin, 0.5 part of calcium gluconate. The suspension is exposed to slow shaking with the caution to complete 10-12 times a 360° tube revolution, and it is left to rest for about 15min [16]. Immediately before preparing the PG, the total volume was adjusted to a level suitable for use in the bone grafts. The mixing of 30ml of PC with 10ml of thrombin solution and 5ml calcium gluconate was acceptable.

C- Post-operative follow-up: The grafted defect will be evaluated by:

Clinical follow-up:

For aesthetics, functions and healing problems with mechanical examination at 12 weeks post-operatively.

Radiological evaluation:

Dental CT with axial 1mm thickness cuts and 1mm intervals are taken through the maxilla, then reformatted panoramic and cross-sectional images

are obtained. 3D facial reconstruction is also carried out with dento-osseous window settings. This 3 minutes procedure is totally non invasive, and doesn't require injection of contrast material.

Dental CT examination will be done preoperatively and postoperatively at 3,6&9 months interval. Parameters that would be assessed included:

Bone defect volume (preoperatively):

- Graft volume, by multiplying bucco-lingual, mesiodistal, and vertical dimensions.
- Overall bone density, by measuring average Hounsfield unit 17 of the area of the graft in at least three different axial levels.
- Gap thickness, between the grafted area and the adjacent normal bone.
- Any post operative complications such as infection, collection, oro-nasal fistula.

RESULTS

This study involved 30 patients with upper jaw defects; 15 patients underwent iliac bone grafting with platelet-rich plasma (study group), and the other 15 patients constituted the control group. In each group, 6 (40.0%) patients had post-tumor resection defects, 8 (53.3%) patients had an alveolar cleft defect, and one (6.67%) patient with a post-traumatic defect. A mixed design ANOVA was calculated to examine the effects of bone graft type (PRP vs. non PRP grafts) and time of measurement (0-month, 3-month and 9-month) on the volume of regenerated bone. A statistically significant (Time x Grafts type) interaction was present; Wilks' Lambda=0.699, $F(2, 27)=5.81$, $p=0.008$, and partial

eta squared=0.301 (large effect size). In addition, the main effect for time was also statistically significant; Wilks' Lambda=0.565, $F(2, 27)=10.39$, $p<0.001$, and partial eta squared=0.435 (large effect size). However, the main effect for graft type was statistically insignificant; $F(1, 28)=0.074$, $p=0.79$, and partial eta squared=0.003 (small effect size). Then, the volume of regenerated bone was influenced by the overall effect of time and graft type.

Fig. (1) shows the time trend in the volume of regenerated bone over the 9-month postoperative follow-up period in PRP & control group. Both groups showed a decrease in the volume of regenerated bone with a peak decrease at 9-month.

A mixed design ANOVA was calculated to examine the effects of bone graft type (PRP vs. non PRP grafts) and time of measurement (0-month, 3-month and 9-month) on the density of regenerated bone. There was no statistically significant (Time X Graft type) interaction was present; Wilks' Lambda=0.94, $F(2, 27)=0.85$, $p=0.44$, and partial eta squared=0.06 (small effect size). The main effect for graft type also was not statistically significant $F(1, 28)=2.42$, $p=0.131$, and partial eta squared=0.08 (moderate effect size). However, the main effect for time alone was statistically significant; Wilks' Lambda=0.009, $F(2, 27)=1.56$, $p<0.001$, and partial eta squared=0.99 (large effect size). Bonferroni post-hoc test for multiple comparisons showed that there was a significant change in the mean density of regenerated bone over time without regard to the graft type (Table 3).

Table (1): Comparison of different diameters of regenerated bone between bone grafting with Platelet-Rich Plasma (PRP) & control group.

Diameters of regenerated bone	Mean (SD)		Mean difference* (95% CI)
	PRP Group (n=15)	Control Group (n=15)	
<i>Bucco-Lingual (mm):</i>			
0 month	8.33 (3.12)	9.73 (4.67)	2.07 (5.05, 0.92)
3-month	7.93 (3.04)	8.27 (3.58)	0.33 (2.81, 2.15)
9-month	7.20 (2.57)	6.60 (2.44)	0.60 (1.28, 2.48)
<i>Mesio-Distal (mm):</i>			
0 month	8.60 (4.44)	9.67 (4.22)	1.07 (4.31, 2.17)
3-month	8.13 (4.07)	7.93 (3.12)	0.20 (2.51, 2.91)
9-month	7.33 (3.77)	6.07 (2.05)	1.27 (1.01, 3.54)
<i>Height (mm):</i>			
0 month	14.80 (5.51)	14.60 (4.82)	0.20 (3.67, 4.10)
3-month	14.20 (5.34)	12.33 (3.66)	1.87 (1.57, 5.31)
9-month	13.47 (5.50)	9.60 (2.50)	3.87 (0.61, 7.13)

SD = Standard Deviation. CI = Confidence Interval.

Table (2): Mixed design ANOVA for the effect of bone graft type and time of measurement on the volume of regenerated bone over a 9-month interval.

Volume of regenerated bone	Mean (SD)		Mean difference* (95% CI)	p-value ^a
	PRP Group (n=15)	Control Group (n=15)		
Volume 1 (0 month)	603.33 (682.05)	812.32 (957.62)		
Volume 2 (3-month)	483.20 (478.98)	339.47 (341.73)	296.49 (72.77, 520.22)	0.007*
Volume 3 (9-month)	363.49 (348.68)	155.17 (123.70)	152.0 (61.92, 242.71)	0.001*

Based on estimated marginal means.

* The mean difference from its preceding measurement is statistically significant at $p < 0.05$.

^a Adjustment for multiple comparisons: Bonferroni test.

PRP = Platelet-Rich Plasma, CI = Confidence Interval.

Table (3): Mixed design ANOVA for the effect of bone graft type and time of measurement on density of regenerated bone over a 9-month interval.

Bone density	Mean (SD)		Mean difference* (95% CI)	p-value ^a
	PRP Group (n=15)	Control Group (n=15)		
Density 1 (0 month)	100.3 (10.60)	93.3 (9.76)		
Density 2 (3-month)	164.0 (11.53)	150.0 (12.10)	60.17 (52.48, 67.86)	<0.001*
Density 3 (9-month)	525.0 (52.47)	523.0 (16.35)	367.0 (348.02, 385.8)	<0.001*

Based on estimated marginal means.

* The mean difference from its preceding measurement is statistically significant at $p < 0.05$.

^a Adjustment for multiple comparisons: Bonferroni test.

PRP = Platelet-Rich Plasma, CI = Confidence Interval.

Fig. (2) shows the time trend in the density of regenerated bone over the 9-month postoperative follow-up period in Platelet-Rich Plasma (PRP) & control group. Both groups showed an increase in the density of regenerated bone with a peak increase at 9-month.

Table (4) shows that there was a statistically significant difference between the PRP & non-PRP grafts regarding the volume of regenerated bone at 9-month postoperative assessment ($p=0.043$). However, there was no statistically significant difference between them regarding the bone density ($p=0.89$).

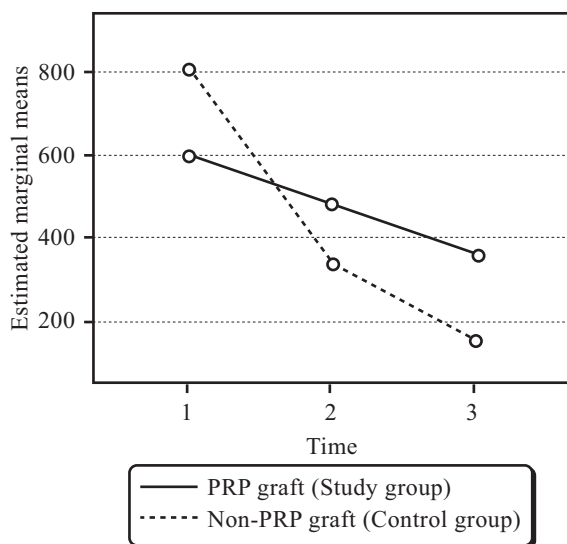


Fig. (1): Time trend for the volume of regenerated bone over the 9-month postoperative follow-up period in Platelet-Rich Plasma (PRP) & control group.

Time 1 = at 0-month, Time 2 = at 3-month, Time 3 = at 9-month.

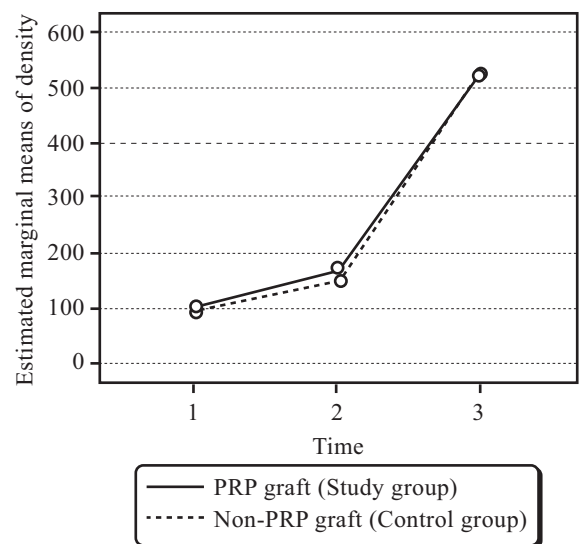


Fig. (2): Time trend for density of regenerated bone over the 9-month postoperative follow-up period in PRP & control group.

Time 1 = at 0-month, Time 2 = at 3-month, Time 3 = at 9-month.

Table (4): PRP & Non-PRP graft.

Characteristics of Regenerated Bone at 9-month	Mean (SD)		Student <i>t</i> -test <i>p</i> -value ^a
	PRP Group (n=15)	Control Group (n=15)	
Volume	363.49 (348.68)	155.17 (123.70)	0.043*
Density	525.0 (52.47)	523.0 (16.35)	0.89

* Statistically significant at $p < 0.05$ and 95% Confidence Level.

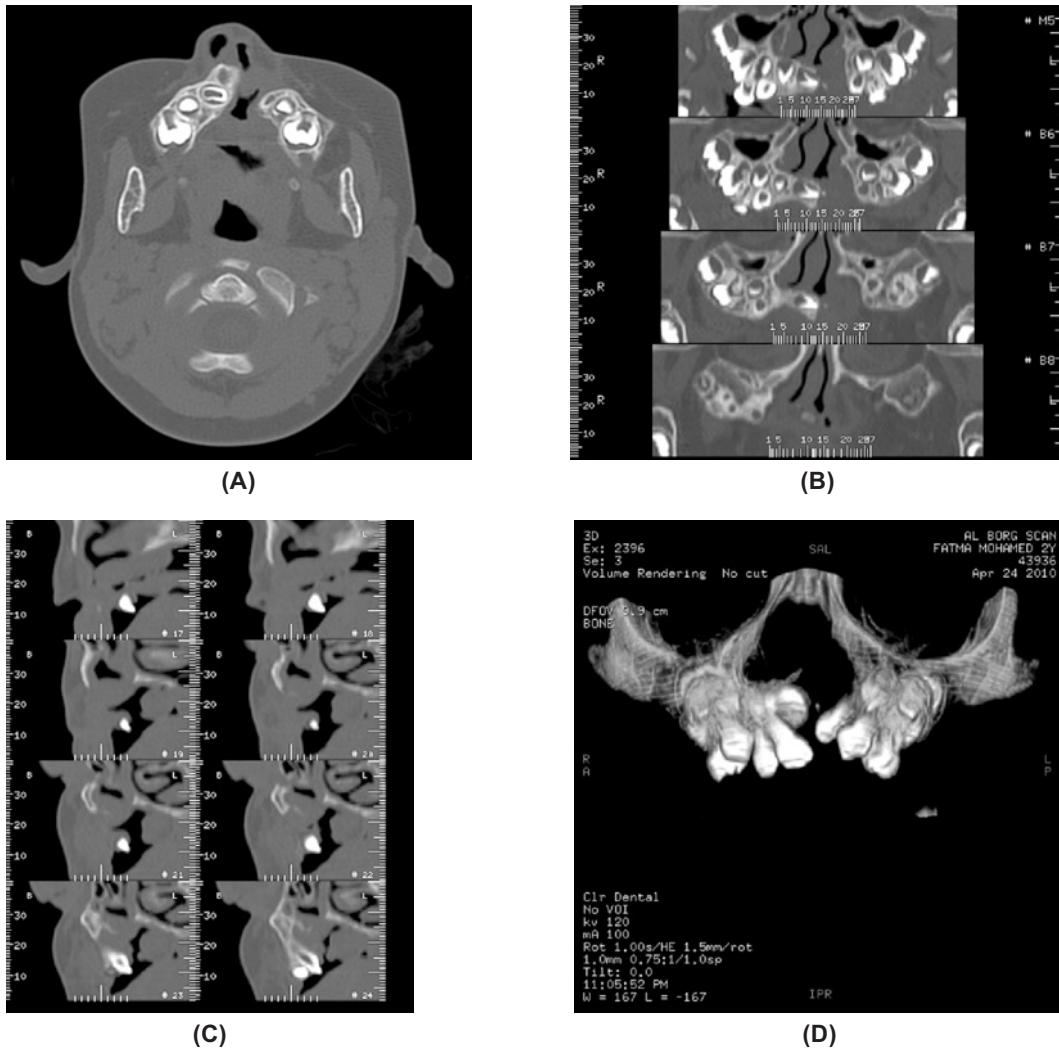


Fig. (3): Pre-operative dental CT study of a cleft alveolus in a 2 year old child. (a): Axial CT of the upper jaw showing a unilateral wide alveolar defect. (b): Reformatted panoramic CT image showing the alveolar defect opening into the left nasal floor. The defect is displayed in both vertical and mesiodistal planes where its height and width are well demonstrated in both planes respectively. Adjacent primordial follicles containing unerupted teeth are visualized. (c): Reformatted cross-sectional CT image showing the alveolar defect in the sagittal (buccolingual) plane. The hard palate posteriorly appears intact. (d): 3D volume-rendered image of the upper jaw demonstrating the alveolar defect.

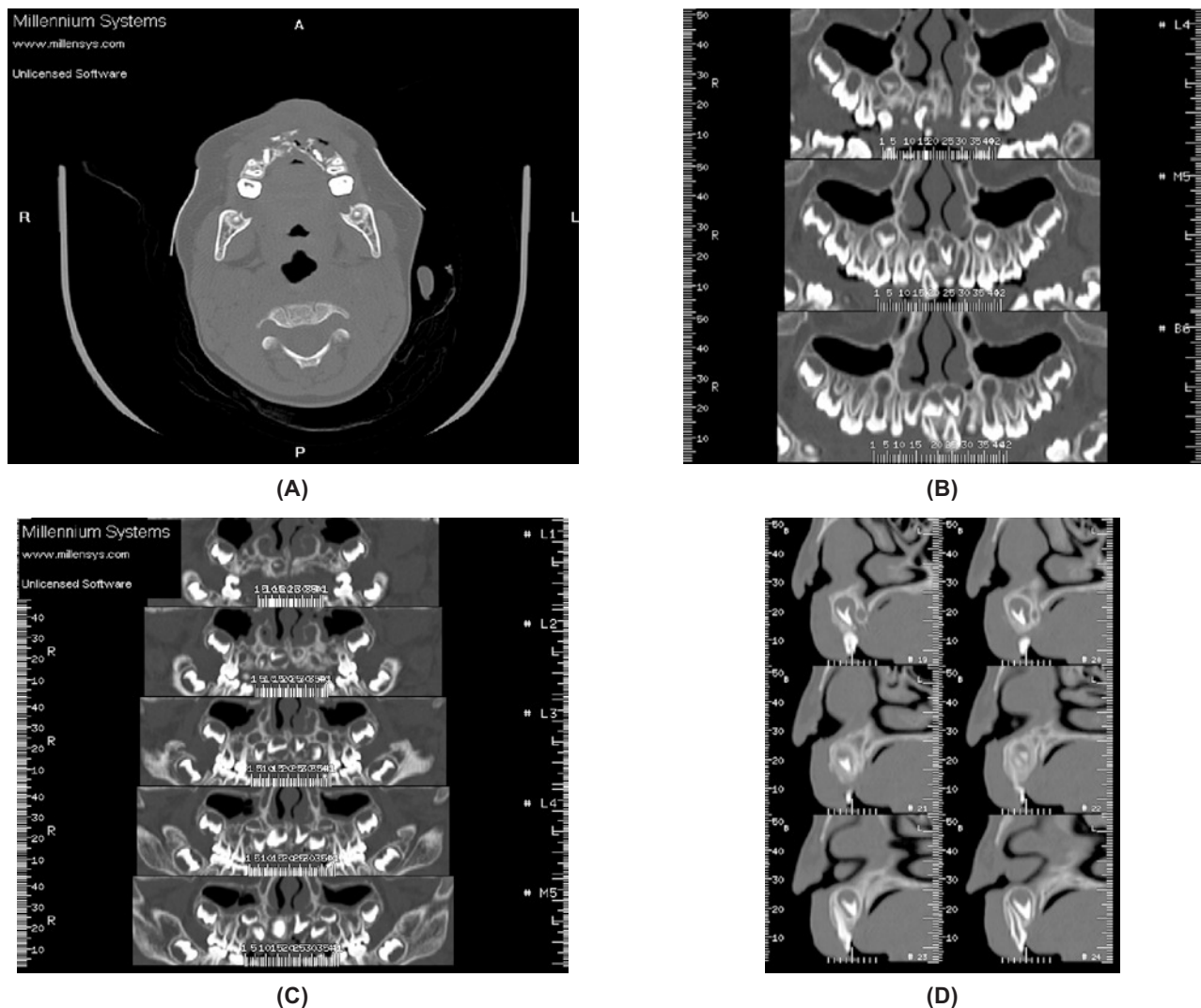


Fig. (4): Post operative dental CT sequential studies of the repaired cleft. (a): Immediate post operative axial CT of the repaired alveolar defect showing plasma-rich graft seen as soft tissue-like density completely filling the defect. (b): Reformatted panoramic CT image (at 6 months interval) of the same patient showing the repaired defect becoming narrower due to healing and bone expansion from both sides of the alveolus. (c): Reformatted panoramic CT image (at 9 months interval) of the same patient showing the repaired defect almost closed with increased overall bone density around it. (d): Reformatted cross sectional CT image (at 12 months interval) of the same patient showing an intact alveolus with normal bone density containing adjacent dental follicles growing into the new repaired alveolar bone.

DISCUSSION

Alveolar bone grafting is not only a significant treatment for different causes of upper jaw defects, but also it may induce the tooth eruption and stabilize the alveolar arch of the maxilla in cleft patients [13].

Iliac cancellous bone is a preferable grafting material because it can be harvested easily and sufficiently and has high osteoinductive potential compared with the other sites. However, even with iliac bone, partial absorption and shortage of reconstructed alveolar height or width may develop postoperatively.

We assumed that PRP might enhance the osteogenesis of autologous iliac bone grafts and lessen postoperative bone resorption, and this study tried to prove this not only by the subjective clinical evaluation, but also by objective radiological parameters as well. These included preoperative measurement of bone defect volume, and 3, 6 & 9 postoperative measurement of the graft volume & density as well as any gap between the graft and the adjacent normal bone if present.

About the volume of the regenerated bone, our results revealed that there was no significant difference by the graft type itself except if mingled with the time of measurement, which revealed

significant interaction. So, the volume of regenerated bone was affected significantly by the overall effect of time and graft type. However, there was no statistical difference between time and graft type interaction on bone density of the regenerated graft.

Schmitz and Hollinger [18] doubt the effects of PRP because platelet-derived growth factor is inhibitory to osteoblastic cells if delivered in a continuous form and increases bone resorption. In our study, it was revealed that PRP could enhance osteogenesis much more than osteoresorption in a remodeling phase within 9 months after the operation. However, it is unknown for how long (>9m) PRP exerts an influence on the bone volume in this study.

Without functional stress in the graft, atrophic bone resorption would occur in the long term [14]. None of our patients have implants yet; therefore, it remains to be proved whether PRP makes a significant difference in subsequent implant treatment.

Marx et al. [13] reported successful results of the reconstruction of the mandibular segment by using PRP. They assessed the bone density on X-ray films in a qualitative analysis. Several kinds of quantitative assessment based on CT scans have been also reported, such as orbital measurements in enophthalmos, [19] intracranial volume in craniosynostosis, [20] and volume of mandible after distraction. However, they did not assess the bone density this time [21]. Our method was theoretically similar to them and simpler by means of the use of reformatted CT software, that enabled us to do sharp quantitative and qualitative evaluation of the bone graft.

Conclusion: PRP was a safe and cost-effective source for growth factors and was easy to extract. It could enhance the osteogenesis of alveolar bone grafting in patients with different upper jaw defects and may be useful for subsequent orthodontic therapy.

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