Efficacy of Platelet-Rich-Plasma (PRP) in Bone Regeneration after Cyst Enucleation in Pediatric Patients – A Clinical Study

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Background: *Platelet-rich-plasma (PRP) is a type of natural source of autologous growth factors, and has been used successfully in various fields. However, the use of PRP in children is not well documented.* ***Objective:*** *To evaluate the efficacy of adding PRP to a bone graft in the bone regeneration of cystic bony defects following cystectomy.* ***Study design:*** *Study sample included 20 children who were randomly divided into two groups with 10 patients in each group; all underwent cystectomy. In the first group (test group), after cystectomy the cystic defect was filled with PRP and bone graft. In the second group (control group) bone graft alone (without PRP) was used. Radiographs were recorded at 1 month, 2 months, 4 months and 6 months intervals after surgery to evaluate the defect bone fill in both groups.* ***Results:*** *The post-operative successive radiographs in the test group showed a significantly greater regeneration of bone in the height of bony defects with application of PRP to bone graft as compared to the control group. In the test group, by the first post operative month, about 58% of the defect was filled, which gradually increased in each month and showed about 94% of defect-fill by 6 months. In the control group, similar observation revealed only 31% of defect-fill by the first post-operative month and a 47% defect-fill at 6 months.* ***Conclusion:*** *The addition of PRP to bone graft appeared to enhance bone regeneration considerably. The combination of PRP and bone graft might have a potential for routine clinical use for regeneration of cystic bony defects in children.*
Keywords: *Platelet Rich Plasma, Bone Regeneration, Bone Graft, Growth Factors, Cystic Defect, Children.*
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INTRODUCTION

Regeneration has emerged as one of the goals of therapy and has become a major area of research. Bone is a specialized form of connective tissue that provides support and protection for vital structures. Regeneration of the lost bony tissue as a result of disease process has long been a desirable goal of surgical therapy in children. Even though bone has a good healing capacity compared to other tissues, the regeneration potential is limited in the cases of large defects such as, after enucleating cysts or impaired healing capacity of the host. It has been shown that after enucleating a cyst, the surrounding jaw bone tends to recede and undergo atrophy.[1] This condition has been shown to have a negative impact on the adjacent teeth, create problems with chewing, cause muscular collapse with development of facial lines, and create difficulties for future implant placement. In such cases, the use of bone grafts, derivatives, or bone substitutes is indicated to promote healing and bone regeneration. In recent years, the process of bone regeneration has been greatly enhanced by the identification of growth factors and the technologic means to use them. The availability to enhance the regenerative process of the human body by utilizing a patient's own blood is something that is now available and is substantiated in the literature.[2] The strategy is to accelerate and enhance the effect of growth factors contained in platelets, the initiators of bone regeneration. This can be done today by the use of autologous platelet rich plasma. The application of growth factors, such as platelet derived growth factor (PDGF) and transforming growth factor (TGF) beta, is seen as an available proposition for enhancing the rate of bone

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formation and the final quantity of bone formed.[3,4] There is enough evidence to suggest its use for oral bone grafting procedures because the addition of PRP to bone grafts has produced a radiographic maturation rate, 1.62 to 2.16 times that of grafts without PRP in adults. As assessed by histomorphometry there was also a greater bone density in grafts in which PRP preparation was added.[5]

A recent innovation in pediatric dentistry is the preparation and use of an autologous PRP as promoters of wound healing and bone regeneration. Hence, the present study was undertaken to assess the role of PRP with bone graft in regeneration of osseous defects of jaws caused by cysts enucleation in young children.

This study evaluated the efficacy of autologous PRP added to a bone graft for regeneration of bone in cystic lesions following surgery.

METHODOLOGY

The present study included 20 children between 7 and 14 years of age, who needed cystectomy for some kind of pathology in the oral cavity. The 20 patients were randomly divided into two groups, with 10 patients in each group. In the first group (test group), after cystectomy, PRP mixed with bone graft (Ortograft) was used for bone regeneration. In the second group (control group), bone graft alone (Ortograft) without PRP was used to fill the defects. Radiographs were taken at 1 month, 2 months, 4 months, and 6 months in both groups to assess the rate of bone regeneration (defect bone fill).

A thorough clinical examination and routine hematological examinations were carried out prior to surgery. Children with systemic diseases, renal disorders, regional malignancies, and respiratory problems were excluded from the study. The nature of the study was explained to the parents of each patient and informed consent was obtained. The protocol and consent form were approved by the institutional ethics committee to conduct the study.

PRP Preparation [3, 6-8]

For the test group, prior to the start of the surgery, 10 ml of blood was drawn intravenously from children and collected in sterile plastic vaccutube coated with anti-coagulant EDTA. Automated centrifugation machine was used for obtaining PRP with a speed of 1300 rpm for 10 min. After centrifugation, 3 layers were obtained:

1) An upper straw colored fluid – PPP (Platelet Poor Plasma). 2) A middle buffy coat rich in platelets. 3) A lower layer rich in RBC.

The straw colored plasma was collected along with buffy coat and 1ml of the RBC layer. This was centrifuged at 2000 rpm for 10 min. The PRP was obtained in the form of a red button at the bottom of the test tube. This was collected with the help of a pasture pipette and transferred into a sterile tube. The platelet poor plasma (PPP) was discarded. PRP obtained after second centrifugation was placed in a sterile tube. For activation, 6 ml of calcium chloride and thrombin were added to PRP. PRP gel was obtained and this was mixed with Ortograft [BIO ceramic composite material, 90% hydroxyapatite and 10% B-Tri-calcium phosphate (Avanthi Laboratories, Hyderabad, India)] in a volume preparation of 1:1. For the test group, PRP mixed with Ortograft was grafted into the cystic cavity; for the control group, Ortograft alone (without PRP) was used after cyst enucleation.

Post-operative evaluation

Post-operative radiographs (panoramic/occlusal/intraoral periapical) were recorded at the end of the 1st, 2nd, 4th and 6th months in both groups. In some patients, radiographs were recorded even 1 year after surgery. All these radiographs were compared with preoperative radiographs to

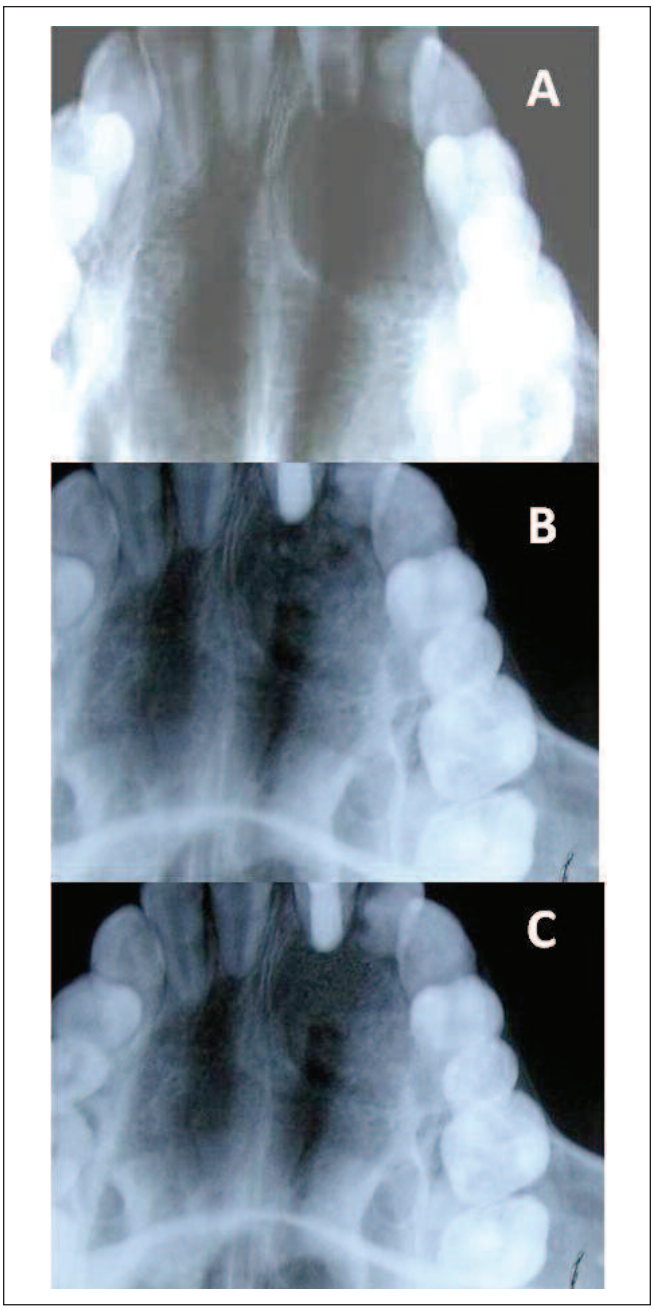

Figure 1: Case 1 (Test group): Radiographs recorded prior to PRP application (A), after 2nd month (B), after 6th month (C)

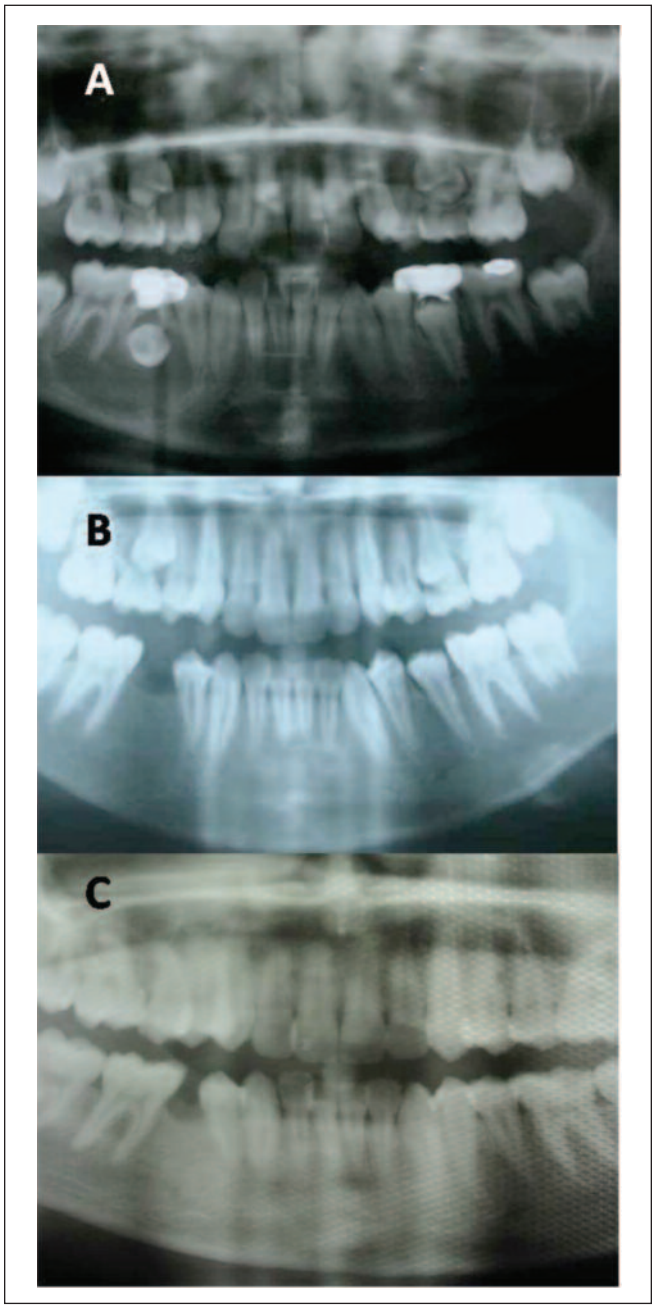

Figure 2: Case 2 (Test group): Radiograph prior to PRP application (A), after 6th month (B), after 1 year (C)

check the height of bone regenerated (defect fill) from the base of the defect to cemento-enamel junction (CEJ) of the adjacent teeth for both groups (Figures 1, 2, 3 and 4). The data obtained from analyzing the radiographs were entered in the master chart and variable parameters were subjected to statistical analysis using Chi-square test.

RESULTS

It was observed that the mean pre-operative defect size in test group was 22.5 mm with standard deviation of ± 4.5 radiographically when calculated from the base of the defect to the CEJ of the adjacent tooth. In the first month, the defect size reduced to 9.5 ± 1.0 mm, the difference from the pre-operative radiograph was 13.0 ± 4.1 mm, and the size of the defect was filled by 58%. In the second month the defect size reduced to 8.9 ± 0.8 mm, the difference from the pre-operative radiograph was 13.6 ± 4.0 mm, and the size of the defect was filled by 60%. In the fourth month, the defect size reduced to 4.9 ± 1.4 mm, the difference from the pre-operative radiograph was 17.45 ± 4.5 mm, and the size of the defect was filled by 78%. In the sixth month, the defect size reduced to 1.3 ± 2.1 mm, the difference from the pre-operative radiograph was 21.2 ± 4.6 mm, and the size of the defect was filled by 94% (Table 1).

In control group (Table 2), the mean pre-operative defect size was 21.4 mm with standard deviation of ± 3.5; Table 2 shows the height of regenerated bone (defect bone fill) at different intervals.

The post-operative successive radiographs showed adequate consolidation (regeneration) of the bone, as manifested by homogeneous radiopacity in test group (Figures 1, 2 and 3) as compared to the control group (Figure 4). On observation for comparison of height of regenerated bone between groups, it was noticed that, in the test group, by first post operative month about 58% of the defect was filled; this gradually increased in each month and showed about 94% of defect fill at 6 months. In the control group, similar observation revealed only 31% of defect fill by the first post operative month and 47% defect fill at 6 month (Table 3). This was found to be statistically significant ($P < 0.001$).

Table 1. Observation for the height of regenerated bone (defect bone fill) with PRP- Bone graft application seen on radiograph

	Bony defect (mm) Mean ± SD	Difference operative defect size (mm)	Defect bone fill
Pre operative	22.5 ± 4.5		
1st month post operative	9.5 ± 1.0	13.0 ± 4.1	58 %
2nd month post operative	8.9 ± 0.8	13.6 ± 4.0	60 %
4th month post operative	4.9 ± 1.4	17.5 ± 4.5	78 %
6th month post operative	1.3 ± 2.1	21.2 ± 4.6	94 %

Table 2. Observation for the height of regenerated bone (defect bone fill) with only Bone graft (without PRP) application seen on radiograph

	Bony defect (mm) Mean ± SD	Difference operative defect size (mm)	Defect bone fill
Pre operative	21.4 ± 3.5		
1st month post operative	15.6 ± 0.9	6.9 ± 2.1	31 %
2nd month post operative	14.9 ± 1.1	7.6 ± 3.0	34 %
4th month post operative	13.3 ± 1.6	9.2 ± 2.5	41 %
6th month post operative	12.0 ± 1.8	10.5 ± 2.6	47 %

Table 3. Comparison of defect bone fills at different intervals between groups

Groups	1st month	2nd month	4th month	6th month
Test (n=10)	58%	60%	78%	94%
Control (n=10)	31%	34%	41%	47%
P-value	0.001	0.001	0.001	0.001

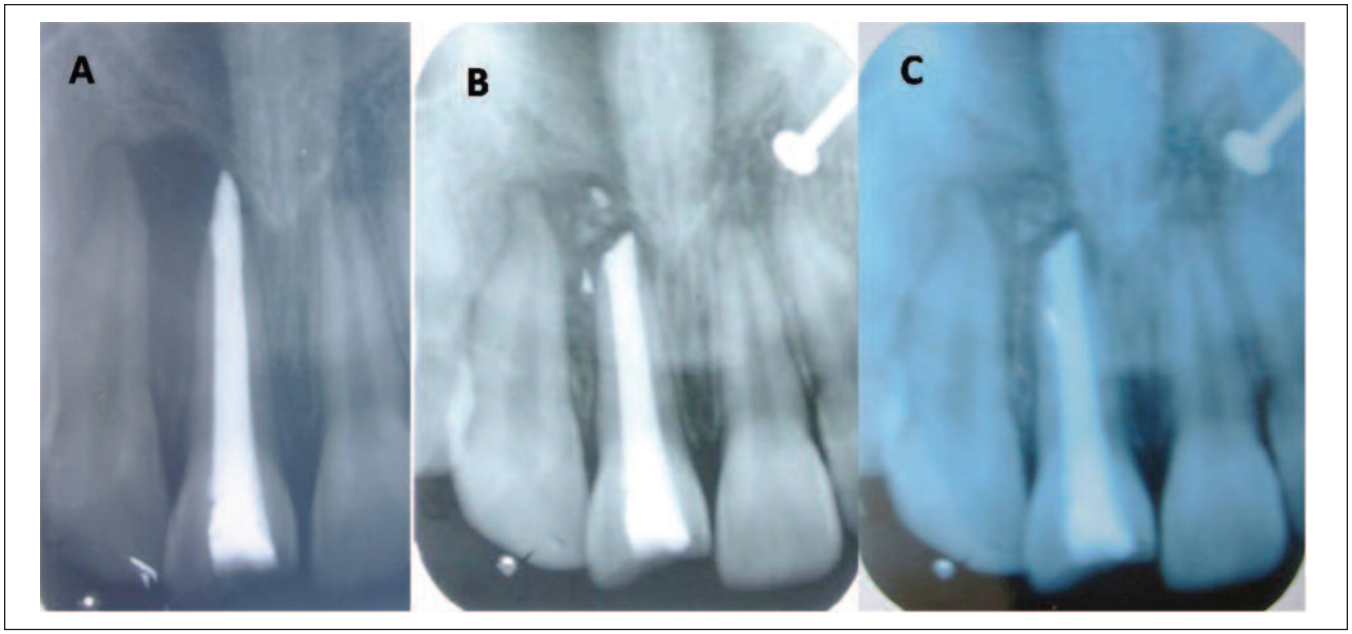

Figure 3: Case 3 (Test group): Before PRP application (A), after 2nd month (B), after 6th month (C)

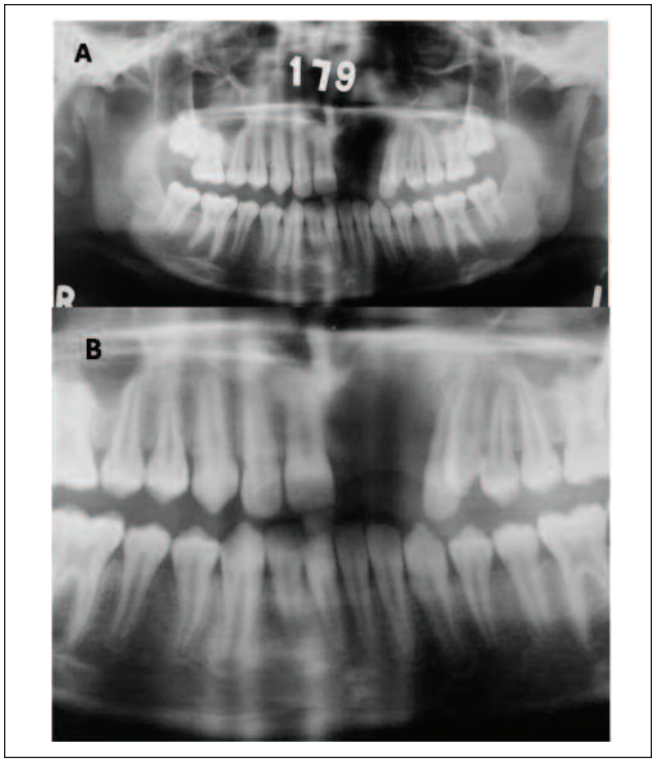

Figure 4: Control group - preoperative radiograph (A), and after 6th month (B)

In one patient (test group), surgical re-entry was performed after 6 months; this revealed complete bone fill of the cystic defect (Figure 5). The quality of the new bone formed was also excellent (dense bone).

DISCUSSION

Because of factors involving PRPs availability and cost, it has become an increasingly popular clinical tool as an alternative source of growth factors for several types of surgery, including oral bone regenerative procedures. PRP was first introduced by Whitman *et al* [9] in 1997. It is an autologous concentrate of platelets suspended in plasma. It is well known that platelets have many functions beyond that of simple hemostasis. Current literature says that platelets contain important growth factors that, when secreted, are responsible for increasing collagen production, recruiting other cells to the site of injury, initiating vascular in-growth, and inducing cell differentiation.[3,4] These are all crucial steps in early wound healing and bone regeneration.

Cystectomy includes the removal of all inflamed soft tissues and sometimes application of different biomaterials to enhance new bone formation in the defect site. Various bone grafts and barrier membranes can be used to achieve optimal healing and regeneration of the cystic cavity. All these approaches are known as regenerative therapies. A recently developed procedure for bony defects utilizes PRP, a concentrated suspension of growth factors.[5] A combination of growth factors, especially PDGR and TGF beta, with bone grafts have been shown to promote bone regeneration in vitro, in animals and in human.[5, 10-18]

Cystic cavity regeneration is a complex process involving both tissue repair and regeneration. The cellular events responsible for healing are controlled and regulated by specific signaling molecules, growth factors, and cytokines. TGF-b1, Bone morphogenetic protein-2 (BMP-2), and PDGF-A are secreted by cells recruited to the healing wound which are released in response to wounding stimuli detected at the cell surface.[1,2] The local availability of these growth factors is enhanced by about threefold or greater in concentration by addition of autologous PRP. Our study used this principle for enhancing the osteoconductive property of

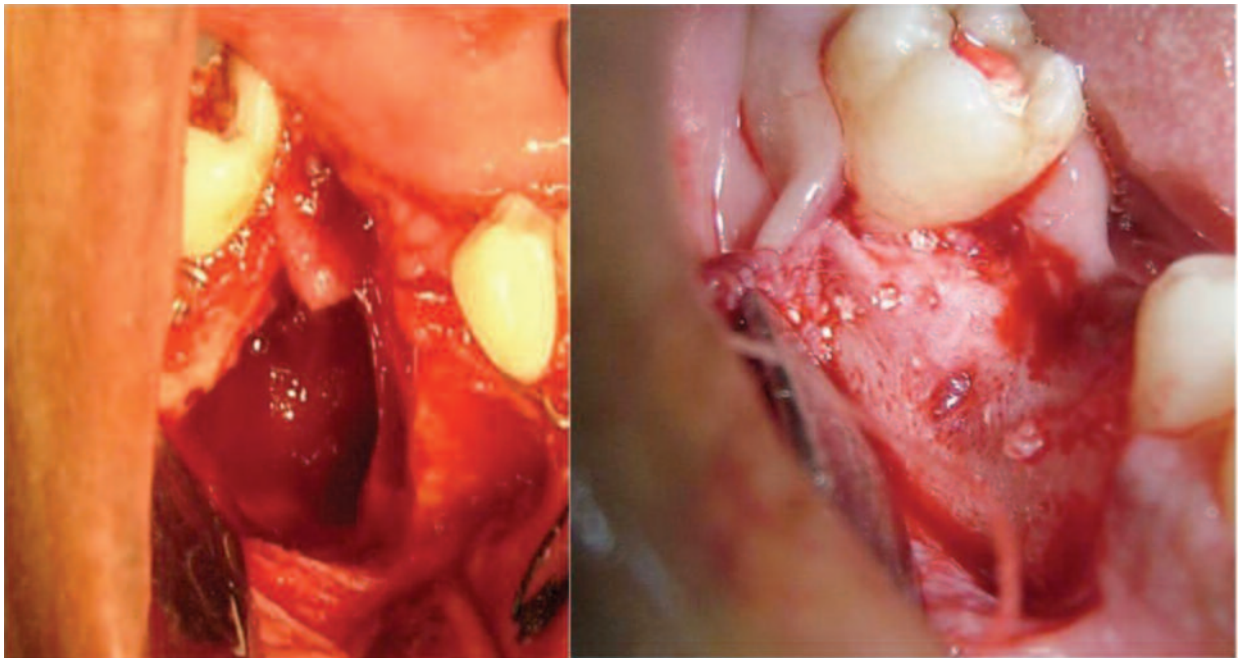

Figure 5: Complete bone fill in cystic defect after surgical re-entry (6 months)

bone graft (Ortograft) by addition of autologous PRP in regeneration of osseous defects of jaws caused by cystectomy.

PRP works via the degranulation of the alpha granules in platelets, which contain growth factors. The active secretion of these factors is initiated by the clotting process of blood when PRP is activated by thrombin. The secreted growth factors immediately bind to their transmembrane receptors on adult mesenchymal stem cells, osteoblasts, fibroblasts, endothelial cells and then cause cellular proliferation, matrix formation, osteoid production and collagen synthesis through cellular message transforming. PRP also contains 3 proteins in blood known to act as cell adhesion molecules for osteoconduction and as a matrix for bone and connective tissue. These molecules are fibrinogen, fibronectin and vitronectin.[19]

PRP also acts as an anti-inflammatory agent by the production of RANTES/CCL5 (acronym for Regulated upon Activation, Normal T-cell Expressed, and Secreted, a protein classified as a chemotactic cytokine or chemokine). CCL5 is a chemotactic for T cells, eosinophils, and basophils and plays an active role in recruiting leukocytes into inflammatory sites, blocking monocyte chemotactic protein-1 release from monocytes and its concentration of lipoxin A4, suggesting that PRP facilitates healing by controlling the local inflammatory response.[20]

Moreover, once PRP preparation is coagulated, it assumes a sticky consistency due to its high fibrin content. The "sticky characteristic" of PRP preparation works as a hemostatic agent, and stabilizes the graft material and the blood clot. The ability to contain the graft material has been demonstrated to be important for regeneration of bone around teeth. PRP is an autogenous preparation, and is inherently safe and free from concerns over transmissible diseases. In addition, the preparation of PRP is simple and rapid. Because of its sticky characteristic, PRP improves the handling properties of the graft material, and may be helpful to shorten the operating time.[21]

In the present study, radiographic assessments indicated that PRP induced faster new bone growth in the cystic cavities in all the patients treated, as compared to patients without PRP. It was observed that the defect was filled by 58% at the first month and after a time interval of 6 months postoperatively, the defect was filled by 94%, and showed significant increase in vertical height on radiographs, as compared to the control group in which only 47% defect-fill was observed. This was statistically significant. The results of the present study are in accordance with and in support of what has been previously suggested for adult patients.[9-12,14,18] The use of PRP facilitated clinical handling of the graft material, which is consistent with previous reports.[9-12]

Researchers have shown radiographically that adding PRP to graft material significantly accelerates the rate of bone formation and improves trabecular bone density as compared to sites treated with only autogenous graft material.[5, 22] Some evidence even suggests that adding PRP to graft material leads to bone growth that is more dense than native bone, a benefit that would allow earlier implant placement, earlier function, and higher predictability in compromised bone cases.[5] Widespread clinical reports and recent published research also suggest that PRP significantly enhances soft tissue healing; reduces bleeding, edema, and scarring; and decreases a patient's self-reported pain levels postoperatively.[14,16,23] PRP growth factors are particularly attractive

for cases in which conditions typically reduce the success of bone grafts and osseointegration, such as patients with severely atrophic maxilla, patients with osteoporosis and those with prior dental disease and subsequently scarred and altered tissues.

Several case study reports also indicate that PRP mixed with autograft or allograft materials improve regenerative results in severe bone defects.[11,14] In one study, 5 patients presenting with severe localized periodontal bone defects, severe bone loss, and guarded prognoses were treated with demineralized freeze-dried bone allografts plus PRP.[24] In each case, faster-than-usual bone formation was noticed radiographically, with significant amounts observed as early as 2 months postoperatively, and on surgical reentry. New bone was of excellent quality and probing depths were also significantly reduced within 4 to 6 months. In one patient of the present study, also on surgical reentry, mature dense bone filling the whole defect was evident. This is the novelty of the present study.

Sharkawy *et al* [20] have shown successful results in regenerative potential of PRP, suggesting that the administration of growth factors may be combined with tissue-regeneration techniques in the repair of intrabony defects, furcations, and cyst cavities to improve the outcome of these treatments.

Contrary to this, there are reports that suggest that PRP when used alone does not benefit bone regeneration.[25,26] Okuda *et al* [27] have compared PRP combined with a biodegradable ceramic, porous hydroxyapatite (HA) with a mixture of HA and saline in the treatment of human intrabony defects. They concluded that treatment with a combination of PRP and HA, compared with HA with saline, led to significantly more favorable clinical improvement in intrabony periodontal defects.

We believe the present study is probably the first study that shows the efficacy of PRP with bone graft (ortograft) in reconstruction of bony defects after cystectomies in pediatric patients. Except for one case report, all other studies and case reports reported so far are the studies done in adults.[28] Based on the previous report[28] and the results of the present study, we can also say that using PRP in children is safe, efficacious, and cost-effective. We also noticed enhanced post-operative comfort levels of young children by way of low level of postoperative pain and reduced surgical morbidity. Its non-toxic nature, ease of availability, and FDA approval could indeed make PRP a boon to pediatric oral and maxillofacial surgical procedures.[28]

A limitation of the present study is the small size of the samples studied. Within the limits of this study, the treatment with a combination of PRP and bone graft led to a significantly more favorable and faster bone regeneration of the cystic defects compared to bone graft alone. However, further studies are necessary to assess the long-term effectiveness of PRP, and a larger sample size is recommended, including the histo-morphometric analysis to assess the quality of the new bone formed.

CONCLUSION

The addition of PRP to a bone graft resulted in a significantly faster regeneration of bone in the cystic bony defects of children compared to using bone graft alone.

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