WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 7, 1723-1740. Research Article ISSN 2277– 7105

A CLINICAL AND RADIOGRAPHIC COMPARISON OF PLATELET-RICH-FIBRIN AND BETA TRI-CALCIUM PHOSPHATE + HYDROXYAPETITE ALLOPLASTIC BONE GRAFT (OSTEON II ®) IN THE TREATMENT OF PERIODONTAL INTRABONY DEFECTS

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Article Received on
28 April 2020,

Revised on 18 May 2020,
Accepted on 08 June 2020,

DOI: 10.20959/wjpr20207-17823

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ABSTRACT

Aim: The purpose of the study was to compare clinically and radiographically the efficacy of Platelet-rich-fibrin and 70%βTri-calcium phosphate + 30% Hydroxyapatite alloplastic bone graft (Osteon II ®) in the treatment of periodontal intrabony defects. **Materials & Method:** 30 surgical sites were selected in 15 patients and treated with either open flap debridement (OFD) with PRF or OFD with 70%β-TCP+30%HA bone graft. Clinical parameters were recorded at baseline, 3months and 6 months postoperatively. The bone-fill was evaluated at baseline, 3months and 6 months with the help of a radiographic grid. **Result:** Significant probing pocket depth (PPD) reduction, clinical attachment level (CAL) gain, significant change in Gingival Recession(GR) and significant bone fill(BF) was observed in both PRF and 70% β-TCP+30%HA bone graft treated sites. **Conclusion:** The use of PRF or 70% β-TCP+30%HA bone graft was effective in the treatment of intrabony defects with an uneventful healing of the sites.

KEYWORDS: PRF, β-TCP, Intrabony defects, Regeneration, Bone Fill.

INTRODUCTION

Periodontal disease is characterized by the presence of gingival inflammation, periodontal pocket formation, and loss of connective tissue attachment and alveolar bone around the affected teeth.[1] The aim of periodontal therapy is to protect and maintain the patient's natural dentition over their lifetime for optimal comfort, function and aesthetic appearance.[2] One of the goals of periodontal therapy is to regenerate the lost periodontal supporting tissues, a process which should include regeneration of multiple tissues including cementum, periodontal ligament, and bone.Different modalities have been proposed to obtain regeneration of periodontal tissues employing various bone grafts, bone substitute materials, guided tissue regeneration (GTR), combination of bone grafts or bone substitutes with GTR and growth factors such as PRF, PRP etc.

Platelet-rich fibrin (PRF) is an autologous concentration of platelets in plasma.It has the characteristic of polymerizing naturally and slowly during centrifugation. The slow polymerization modeconfers to the PRF membrane, a particularly favorable physiologic architecture to support the healing process.[3] The effect of platelet-rich fibrin on human periodontal ligament fibroblasts and application in periodontal infrabony defects was studied by Chang et al. and reported that PRF was found to increase extracellular signal-regulated protein kinase phosphorylation and osteoprotegerin in periodontal ligament fibroblasts and upregulation of alkaline phosphatase activity.[4]

Apart from PRF and PRP; bone grafts are also one of the most common treatment modality used for regenerative therapy. Alloplastic materials are bone grafts which are used as bone substitutes. They are biologically acceptable, allowing bone ingrowths and bone remodeling while maintaining volume.[5] Additionally, alloplastic materials have several advantages, such as (a) the lack of required donor site, (b) ample supply, and (c) no risk of disease transmission.[6] Among the various alloplastic materials available today, calcium phosphate ceramic is widely used for periodontal regeneration. Few materials like hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) have shown significant clinical improvement at grafted sites compared to non-grafted sites in controlled clinical studies.

Commercially available OSTEON II® is a newly developed alloplastic material containing 30% HA and 70% β -TCP. Particle size is from 0.2–2.0mm. A study was done by F. Schwarz et al to investigate bone regeneration following application of hydroxyapatite + beta tricalcium phosphate (BCG) or a collagen-coated natural bone mineral (BOC) in combination

with a collagen membrane at dehiscence-type defects in dogs. It was seen that both BCG(60% HA + 40% b-TCP) and BOC may provide an osteoconductive scaffold to support guided bone regeneration procedures at dehiscence-type defects.[7] According to a study done by Cristina Bucchi et al, the authors used Osteon® 70/30, Osteon collagen®, and Osteon II®. And it was seen that the Osteon® 70/30 graft showed the highest radiopacity values in the 3-month period.[8] Thus, there are many studies that compares the effectiveness of PRF and different composition ofHA and β –TCP[8-10] but there are paucity of studies that compares the effectiveness of PRF and 30% HA + 70% β -TCP in intrabony defects. Hence this study aims to evaluate and compare the treatment of intrabony defects by using 30% HA+ 70% β -TCP and PRF.

MATERIAL AND METHODS

A clinical and radiographic comparative evaluation of platelet-rich-fibrin and beta tri-calcium phosphate + hydroxyapatite alloplastic bone graft (Osteon II ®) in the treatment of periodontal intrabony defects studywas carried out in the Department of Periodontology D.A.P.M.R.V. Dental College Bangalore. The study population was selected from the subjects visiting the out-patient section of the Department of Periodontics, D A Pandu Memorial R V Dental College, Bangalore. Subjects were selected from those diagnosed as having chronic periodontitis, with clinical and radiographic evidence of intrabony defects and indicated for regenerative periodontal surgery.

INCLUSION CRITERIA included Patients diagnosed with chronic periodontitis (AAP, 1999), Age between 35 and 65 years, Interproximal probing depth ≥ 5mm following phase I therapy, The sites exhibiting clinical and radiographic evidence of intrabony defects ≥3mm deep.

EXCLUSION CRITERIA included Subjects who had received periodontal flap / regenerative therapy within the past 1 year, Pregnant and lactating patients, Patients with uncontrolled diabetes and immuno-compromised patients, Patients who were under antibiotics, analgesics, steroids for over past 3 months, Smokers, Patients who demonstrated poor oral hygiene maintenance, with a gingival index score ≥ 2.1 after 2 weeks of Phase I therapy, Teeth with ≥ Grade II mobility, Patients with platelet count less than 200000/mm3 The Ethical clearance for the study was obtained from the ethical committee and review board of the institution. The participants were explained about the study and a written consent was obtained from each of the participants.

1. Pre-surgical procedures

Case history was recorded on a previously prepared clinical proforma, study casts and clinical photographs were taken. Routine lab investigations, complete hemogram and random blood sugar were done. Phase I therapy included oral hygiene instructions, scaling and root planing using hand and ultrasonic instruments, were performed. Trauma from occlusion, if present, was relieved. Adjunctive chemical plaque control in the form of Chlorhexidine mouthwash 0.2% twice daily was advised. Patients were re-evaluated 2 weeks after phase I therapy. Oral hygiene status was assessed using Plaque Index (Sillness and Loe (1964)) and Gingival index (Loe and Sillness (1963)).

2. Surgical Protocol

Surgical procedure in the selected defects was done after completion of pre-surgical therapy. Pre- procedural mouth rinse was performed with 0.2% chlorhexidine digluconate rinse. The acrylic stent was put in place and probing depth, clinical attachment level and gingival recession was recorded using a University of North Carolina (UNC-15) probe from the fixed reference mark on the stent. Following administration of LA (2% lignocaine hydrochloride with 1 in 80,000 adrenaline), buccal and lingual crevicular incisions were made using a No.15 sterile surgical blade. A full thickness mucoperiosteal flap was reflected using a Molt No.9 periosteal elevator. Care was taken to preserve as much inter-proximal soft tissue as possible. Meticulous defect debridement and root planing were carried out using ultrasonic instruments and area specific curettes (Gracey curettes, Hu- Friedy, Chicago, IL, USA), following which the surgical site was thoroughly irrigated with Povidone Iodine. Before the placement of the graft, a 3-0 non resorbable braided silk suture was passed through the buccal and lingual papillae and the suture was left loose. This was done in order to prevent removal of the graft particles/ PRF by the passage of the needle as well as the suture material. Suturing was done using a half circle, triangular stainless-steel suture needle (Sabre, Needle Industries (India) Private Ltd, Tamilnadu) and 3-0 braided black silk suture material (Lifeline, Sutures manufacturing company, Peenya, Bangalore).

Preparation of PRF: The PRF was prepared following the protocol developed by Choukroun et al for group II patients. The patient's blood sample was drawn. Immediately after the blood draw, the dried monovettes (without anticoagulant) were centrifuged at 3000 rpm for 10 minutes in the tabletop centrifuge. A structured fibrin clot formed in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma at the top (Platelet Poor

Plasma-PPP). PRF was separated from red corpuscles at the base, preserving a small red blood cell (RBC) layer, using a sterile tweezer and scissors just after removal of Platelet Poor Plasma (PPP) and then transferred onto a sterile dappen dish.

Group A patients received 70% beta tri-calcium phosphate + 30%hydroxyapetite alloplastic bone graft (Osteon II ®). The graft material was mixed with saline and the defect was filled till the rim of the defect. In Group B patients, bone defects were filled with PRF.

The suturing was then completed and Non-eugenol periodontal dressing (Coe pack™, GC America Inc., Chicago, IL, USA) was placed.

3. Post-Surgical Care Suitable antibiotic and analgesic were prescribed. Tablet Ciprofloxacin (500 mg) + Tinidazole (600 mg) 1-0-1* 5 days and Tab Aceclofenac (100mg) + Paracetamol/Acetaminophen (325mg) + Serratiopeptidase (15mg) 1-0-1* 3 days). Patients were advised to rinse with chlorhexidine digluconate (0.2%) twice a day for 2 weeks following surgery. Patients were advised not to smoke. Patients were also advised not to brush the surgical site for 7-10 days. Periodontal dressing and sutures were removed 7-10 days after surgery. Surgical wounds were gently cleansed with 0.2% of chlorhexidine digluconate and subjects were instructed for gentle brushing with a soft toothbrush. Patients were advised to refrain from flossing and using of any interdental aids in the area for 4 weeks. Each patient was reinstructed for proper oral hygiene measures at every recall review.

4. Post-Surgical Evaluation and Review

Clinical parameters: Gingival Index (GI) and Plaque Index (PI) were re-evaluated at 3 monthsand 6 months.

Probing Pocket Depth (PPD), Clinical Attachment Level (CAL), Gingival Recession (GR) were also re-evaluated at 3 months and 6 months using the previously used acrylic stents to provide a reproducible insertion axis.

Radiographic parameters: Intraoral periapical radiographs were taken using long cone paralleling technique with radiographic grid in position at 3 months and 6 months to assess the depth of the defect (DOD).

STATISTICAL ANALYSIS- Student Paired t test.

RESULTS

1. **Gender distribution -** Total 15 patients were selected, of which8 were male patients and 7 were female patients.
2. **Age distribution-** Of the selected 15 patients, mean age distribution of male patients were 37 years whereas for female patients were 37.71 years.
3. **Plaque index**

Intra group Comparison- Group A-The difference in the mean PI was found to statistically significant between all the time intervals (P<0.05).

Intra group Comparison- Group B-The difference in the mean PI was found to be statistically significant between all the time intervals (P<0.05).

Inter- group comparison: (Table-1, Graph-1)-Comparison of plaque index scores between two groups at different time intervals has been shown in Table 1, Graph 1. The mean difference in the values between two groups at baseline, 3 monthsand 6 months were 0.02, 0.01, 0.02 and 0.05 respectively. No statistically significant difference was observed between the two groups at different time intervals with respect to mean PI (P>0.05).

4. **Gingival index**

Intra group Comparison- Group A-The difference in the mean GI was found to be statistically significant between all the time intervals (P<0.05).

Intra group Comparison- Group B-The difference in the mean GI was found to be statistically significant between all thetime intervals (P<0.05).

Inter- group comparison: (Table-2, Graph-2)-Comparison of gingival index scores between two groups between different time intervals has been shown in Table 2, Graph 2. The mean difference in the values between two groups at baseline, 1 month, 3 months and 6 months were -0.06,-0.07, 0 and 0.01 respectively No statistically significant difference was observed between the two groups at different time intervals with respect to mean GI (P>0.05).

5. **Probing Pocket Depth**

Intra group Comparison: Group A -The difference in the mean PPD was found to be statistically significant between all the time intervals. (P<0.05).

Intra group Comparison: Group B -The difference in the mean PPD was found to be statistically significant between all the time intervals. (P<0.05).

Inter- group comparison: (Table-3, Graph-3)-The mean difference in the values between two groups at baseline, 3 months and 6 months were 0, -0.2 and -0.93 respectively. No statistically significant difference was observed between the two groups at different time intervals with respect to mean PPD (P>0.05).

6. Clinical Attachment Level (CAL)

Intra group Comparison: Group A -The difference in the mean CAL was found to be statistically significant between all the time intervals. (P<0.05)

Intra group Comparison: Group B -The difference in the mean CAL was found to be statistically significant between all the time intervals. (P<0.05)

Inter-group comparison: (Table-4, Graph-4)-The mean difference in the values between two groups at baseline, 3 months and 6 months were 0.07, 0.14 and -0.47 respectively. No statistically significant difference was observed between the two groups at different time intervals with respect to mean CAL (P>0.05).

7. Gingival Recession

Intra-group comparison: Group A -The difference in the mean gingival recession was found to be not statistically significant between all the time intervals. (P<0.05)

Intra-group comparison: Group B -The difference in the mean gingival recession was found to be not statistically significant between all the time intervals. (P<0.05)

Inter-group Comparison: (Table-5, Graph-5)-The mean difference in the values between two groups at baseline, 3 months and 6 months were0.07, 0.13and 0 respectively. No statistically significant difference was observed between the two groups at different time intervals with respect to mean GR (P>0.05).

8. Depth of The Defect

Intra group Comparison: Group A-The difference in the mean depth of the defect (bone fill) was found to be statistically significant between all the time intervals. (P<0.05)

Intra group Comparison: Group B -The difference in the mean depth of the defect (bone fill) was found to be statistically significantbetween all the time intervals. (P<0.05)

Inter group comparison: (Table-6, Graph-6)-The mean difference in the values between two groups at baseline, 3 months and 6 months were 0.13, 0.27 and 0 respectively. No statistically significant difference was observed between the two groups at different time intervals with respect to mean bone fill (P>0.05).

DISCUSSION

Osteon II is a100% synthetic bone graft material with an effective osteoconduction property. It has better handling properties, excellent wettability. Pore size is 250μm. The Hydroxyapatite particles act to maintain the augmented space, whereas β-tricalcium phosphate promotes bone formation within that space. This controlled activity and balance between resorption/solubilization guarantees the stability of the biomaterial while promoting bone ingrowth.[11] Role of platelets in regeneration was proven way back in the 1970s, owing to the fact that it is a reservoir of growth factors that are responsible for neovascularization, collagen synthesis, cell division, cell differentiation, induction, and migration of other cells to the injured site.[12,13]

A total of 15 patients in the age range of 35-65 years were enrolled for this study, of which 8 were male patients and 7 were female patients. Mix gender within study groups was advocated, since this was considered the best approach to equality for a research.[14] Of the selected 15 patients, mean age distribution of male patients were 37 years whereas for female patients were 37.71 years. The properties of alveolar bone do not remain constant with age; rather, they change throughout life. Hence, in this study, patients with similar age groups were considered to avoid bias.[15]

A Gingival Index score of 2.1 and above after 2 weeks of Phase I therapy was considered in exclusion criteria because it indicated poor oral hygiene and inadequate compliance of the patient. Patients with platelet count less than 200000/mm3 were excluded from study because such patients will have reduced platelet function (because of reduced platelet aggregation and activation). A therapeutic material will not be obtained from such patients.[16,17]

The present study has included only those sites that have shown Interproximal probing depth ≥ 5mm following phase I therapy and radiographic evidence of angular bone loss ≥3mm deep. This was in accordance with study done by Laurell et al who has shown that to benefit from regenerative procedures; depth of defect should be at least 3-4 mm.[18]

Plaque index and gingival index were assessed at baseline, 3 months and 6 months. The results of the study showed a statistically significant decrease in the plaque index and gingival index from baseline to 1 month, baseline to 3 months and baseline to 6 months in Group I as well as in Group II. However no statistically significant difference was recorded between the two groups which suggest that all patients were very well motivated and there

was a good maintenance of oral hygiene throughout the study in both the groups. There was no untoward soft tissue reactions and inflammation seen in both the groups throughout the study period. The improvement in gingival and plaque status could be due to the surgery and frequent reinforcement of oral hygiene maintenance. These results were similar to those of Chandrashekar et al., Prathap et al. and Pinipe et al.[19,20]

Probing Pocket depth (PPD) is another important parameter that is assessed to know the status of the periodontium after regenerative periodontal therapy. Most of the surgical studies show reduction in probing pocket depth. In, this study also significant reduction in pocket probing depth was observedbetween different time intervals. In Group A the values were 8.06±1.62 at baseline, 5.86±1.30 at 3 months, 3.8±0.67 at 6 months and in Group B the values were 8.06 ±1.86 at baseline, 6.06±1.09 at 3 months, 4.73±0.70 at 6 months. There was no significant difference ($P > 0.05$) observed at baseline, 3 months, and 6 months between test and control groups. The results were in accordance with studies done by Lee et al, Ozdemir B et al, Elgendy EA et al.[21-23]

There was significant gain in CAL. In Group A the values were 9±2.59 at baseline, 7.6±2.16 at 3 months, and 4.53±1.8 at 6 months and in Group B the values were 8.93±3.1 at baseline, 7.46± 2.74 at 3 months, 5±2.26 at 6 months. There was no significant difference between the groups at baseline, 3 months and 6 months. This was in accordance with studies done by Sharma A et al andStavropoulos A et al.[24]

The comparable gain in the clinical attachment level could be due to increased bone fill, tissue repair and reattachment. The apparent reduction in pocket depth and CAL might be because of the resolution of gingival inflammation with collagen fibers replacing the inflammatory infiltrate within the tissues. The results of the present study support the role of various growth factors present in PRF in accelerating the soft tissue and hard tissue healing.[25]

Gingival recession was evaluated at baseline, 3months and 6 months. In Group A the values were 1.33±1.34 at baseline, 1.86± 1.40 at 3 months, 1.36 ± 0.88 at 6 months and in Group B the values were 1.26 ± 1.27 at baseline, 1.73± 1.62 at 3 months, 1.36± 1.30 at 6 months. There was increase in the recession after 3 months which reduced by the end of 6 months. But when compared to baseline, recession was slightly more during 6 months evaluation.

These findings are in consistency with studies by Stavropoulos A et al and Sharma A et al.[24] Increase in GR may be because of the shrinkage of gingiva during the healing period.[26]

Another aim of this study was to measure the bone fill in intrabony defects. The bone fill was assessed with the help of radiographic grid which was used along with IOPA at baseline, 3 months and 6 months after regenerative surgery. In Group A the distance from the bone crest to depth of defect was were 5.46±1.4 at baseline, 4.4±1.5 at 3 months, 3.06±1.22 at 6 months and in Group B the values were 5.33±1.91 at baseline, 4.13±1.72 at 3 months, and 3.06±1.57 at 6 months. Hence, significant bone fill was observed in both the groups at 3 months and 6 months compared to baseline.[27,28]

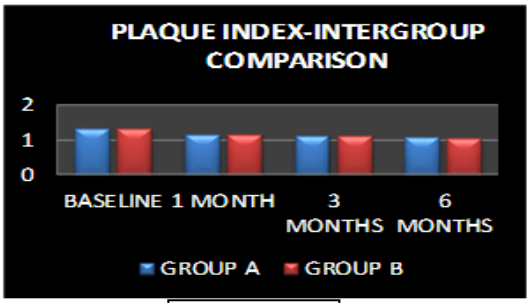
Increase in bone fill can be because of the osteoconductive property of the bone graft where the bone graft material serves as a scaffold for new bone growth that is perpetuated by the native bone and the osteoblasts from the margin of the defect that is being grafted utilize the bone graft material as a framework upon which to spread and generate new bone.

Whereas in case of PRF, permanent and long-lasting polymerization of PRF provides a slow release of the contents (growth factors and cytokines), keeps them contained in the fibrin matrix for a long time and prevents them from undergoing proteolysis.[29,30]

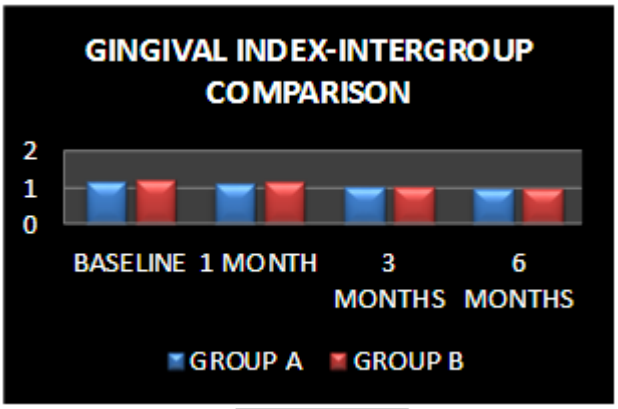
LIMITATIONS OF STUDY

The major limitation of the present study is - The **small sample size** taken which is inadequate to evaluate the efficacy of graft materials in the treatment of human periodontal intrabony defects and the intrabony defects included in our study **differed in their dimension** i.e the width and depth. The treatment outcome is influenced by the differences in the dimensions of the defect. Hence the results of the present study may be influenced by the same.

GRAPHS



GRAPH -1



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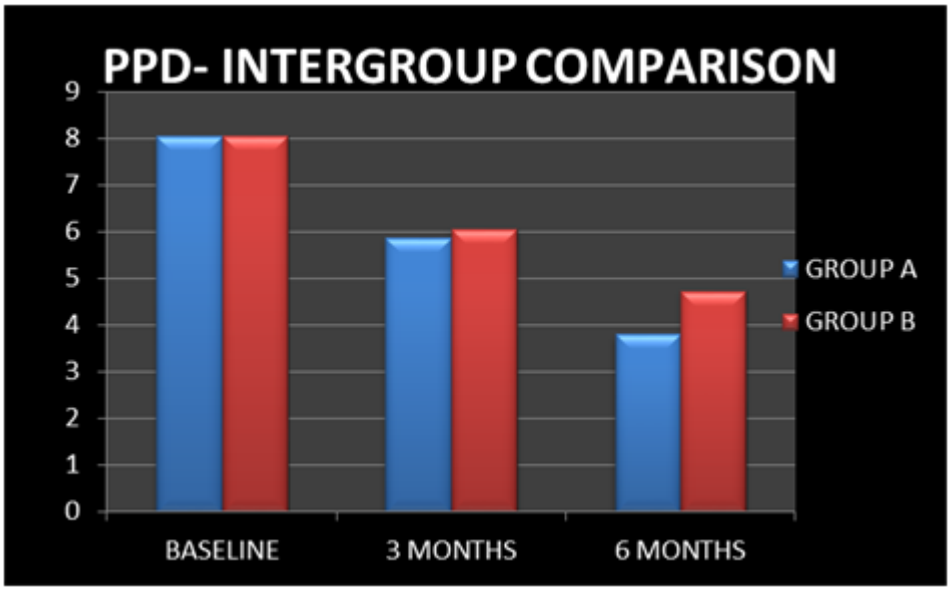

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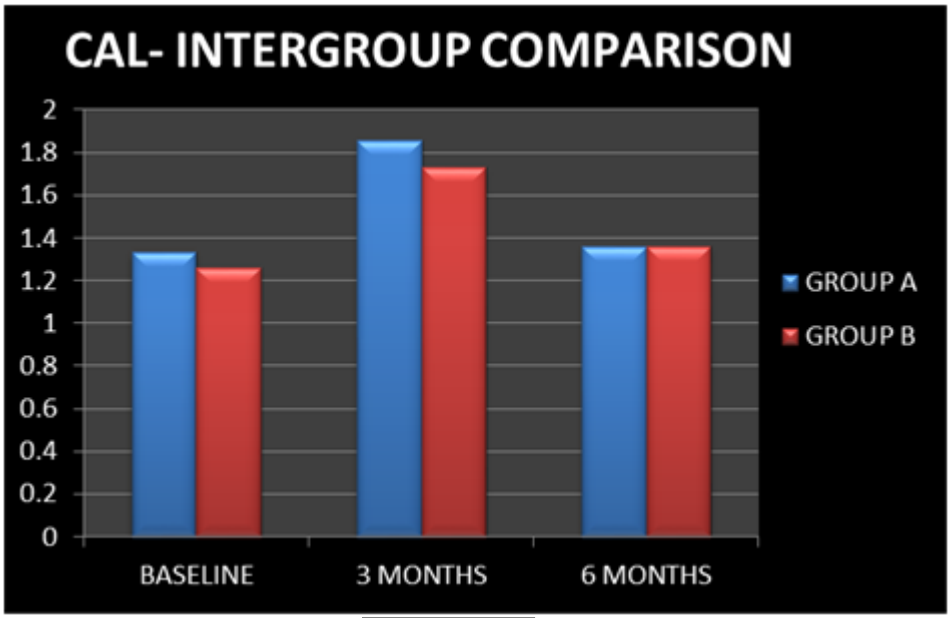

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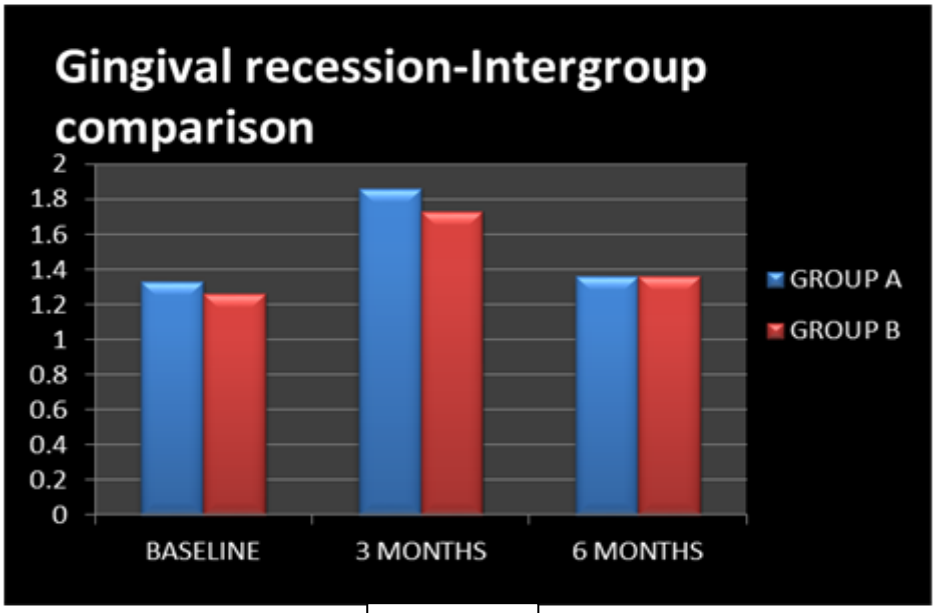

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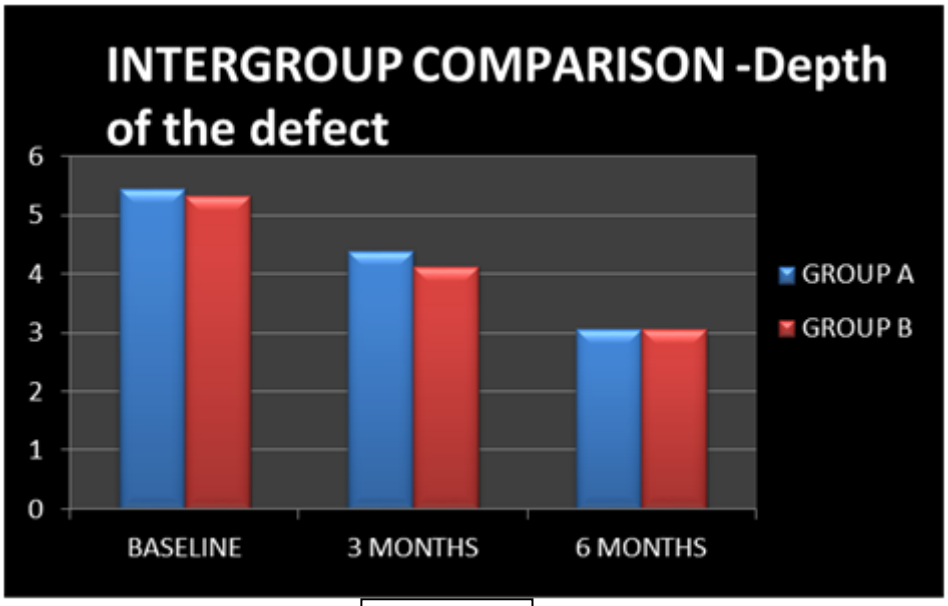


GRAPH -6

Table 1: PLAQUE INDEX- Inter- group comparison.

TIME INTERVAL	GROUPS	Mean	Std. Deviation	Std. Error of mean	Mean Difference	t value	P value
Baseline	GROUP A	1.34	0.16	0.04	0.02	+0.22	0.4505
	GROUP B	1.32	0.16	0.16			
30 days	GROUP A	1.16	0.11	0.02	0.01	+0.17	0.4175
	GROUP B	1.15	0.10	0.02			
90 days	GROUP A	1.14	0.12	0.04	0.02	+0.47	0.3052
	GROUP B	1.12	0.10	0.02			
180 days	GROUP A	1.10	0.12	0.03	0.05	+1.16	0.1268
	GROUP B	1.05	0.09	0.02			

Table 2: GINGIVAL INDEX- Inter- group comparison.

TIME INTERVAL	GROUPS	Mean	Std. Deviation	Std. Error of mean	Mean Difference	t value	P value
Baseline	GROUP A	1.18	0.18	0.04	-0.06	-0.94	0.423
	GROUP B	1.24	0.19	0.05			
30 days	GROUP A	1.1	0.13	0.03	-0.07	-1.43	0.320
	GROUP B	1.17	0.14	0.03			
90 days	GROUP A	1.05	0.20	0.05	0	0	0.50
	GROUP B	1.05	0.20	0.05			
180 days	GROUP A	1	0.16	0.04	0.01	+0.09	0.077
	GROUP B	0.99	0.23	0.06			

Table 3: PPD- Inter-group comparison.

TIME INTERVAL	PPD	Mean	Std. Deviation	Std. Error of mean	Mean Difference	t value	P value
Baseline	GROUP A	8.06	1.62	0.41	0	0	0.3052
	GROUP B	8.06	1.86	0.48			
90 days	GROUP A	5.86	1.30	0.33	-0.2	-0.4544	0.2686
	GROUP B	6.06	1.09	0.28			
180 days	GROUP A	3.8	0.67	0.17	-0.93	-3.7037	0.4437
	GROUP B	4.73	0.70	0.18			

Table 4: CAL Inter-group comparison.

TIME INTERVAL	CAL	Mean	Std. Deviation	Std. Error of mean	Mean Difference	t value	P value
Baseline	GROUP A	9	2.59	0.66	0.07	0.0639	0.2519
	GROUP B	8.93	3.10	0.80			
90 days	GROUP A	7.6	2.16	0.55	0.14	0.1476	0.1918
	GROUP B	7.46	2.74	0.70			
180 days	GROUP A	4.53	1.80	0.46	-0.47	-0.6232	0.2045
	GROUP B	5	2.26	0.58			

Table 5: GINGIVAL RECESSION-Inter-group comparison.

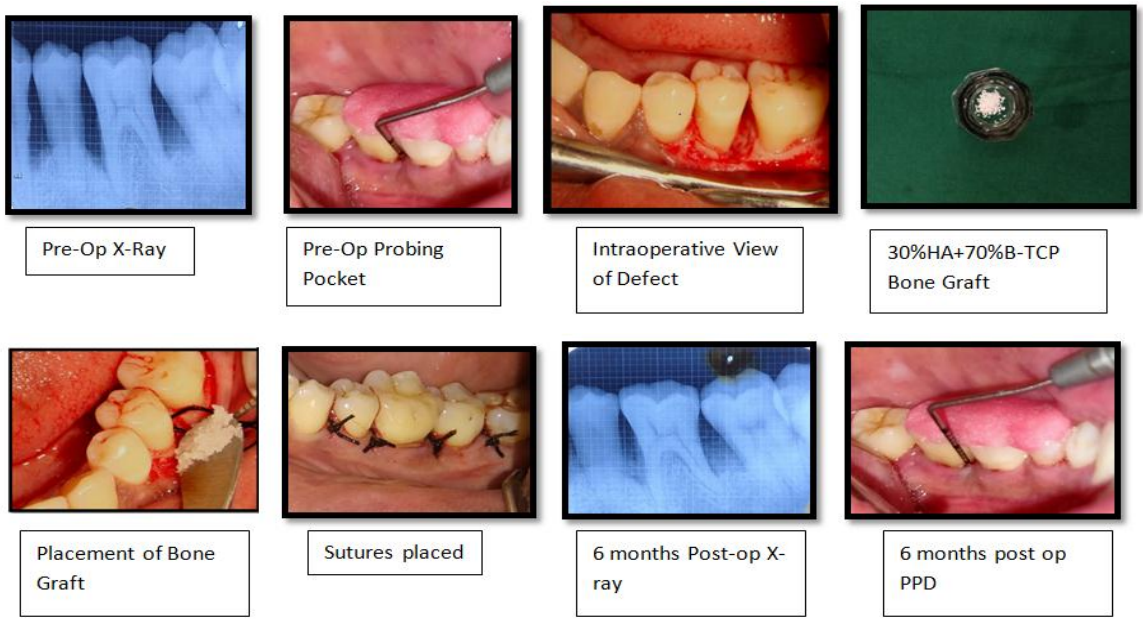
TIME INTERVAL	GR	Mean	Std. Deviation	Std. Error of mean	Mean Difference	t value	P value
Baseline	GROUP A	1.33	1.34	0.34	0.07	0.1391	0.4304
	GROUP B	1.26	1.27	0.33			
90 days	GROUP A	1.86	1.40	0.36	0.13	0.2403	0.3004
	GROUP B	1.73	1.62	0.41			
180 days	GROUP A	1.06	0.88	0.22	0.06	0.1634	0.0761
	GROUP B	1	1.30	0.33			

Table 6: DEPTH OF DEFECT Inter-group comparison.

TIME INTERVAL	GROUPS	Mean	Std. Deviation	Std. Error of mean	Mean Difference	t value	P value
Baseline	GROUP A	5.46	1.40	0.36	0.13	0.22	0.1309
	GROUP B	5.33	1.91	0.49			
90 days	GROUP A	4.4	1.50	0.38	0.27	0.45	0.3052
	GROUP B	4.13	1.72	0.44			
180 days	GROUP A	3.06	1.22	1.31	0	0	0.1742
	GROUP B	3.06	1.57	0.40			

FIGURES

GROUP-A



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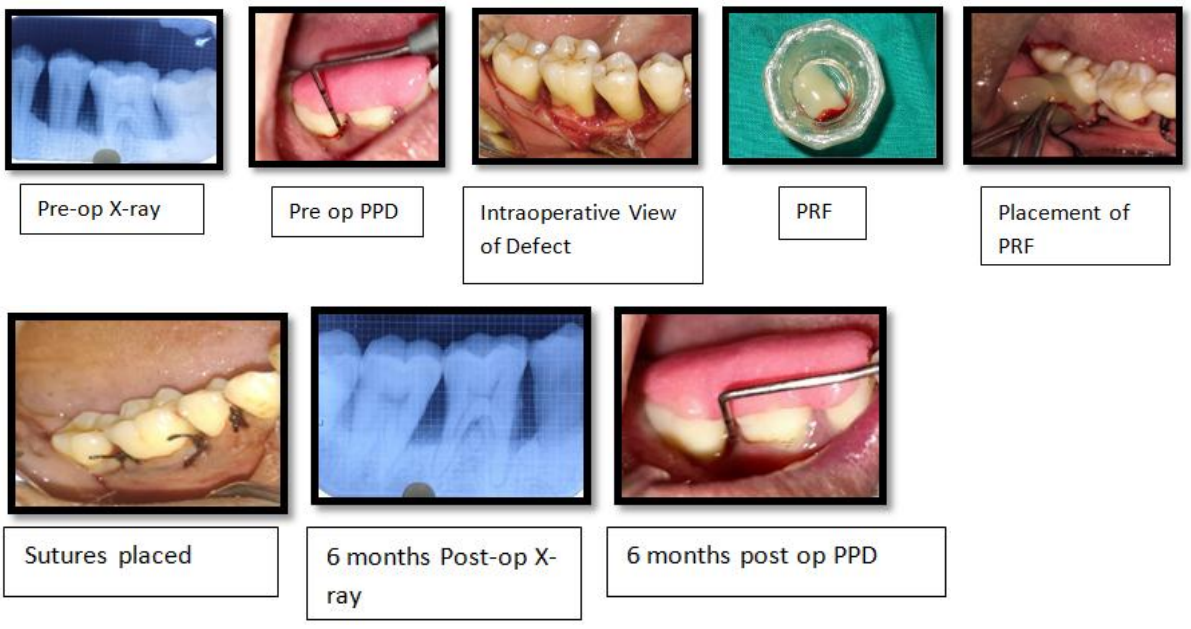

CONCLUSION

Treatment with either PRF or 30% HA +70% β-TCP (Osteon II®) bone graft stimulated a significant increase in the PD reduction, CAL gain, and bone fill in both the study groups. Results obtained from 30% HA +70% β-TCP bone graft were almost similar toPRF group. Hence, both PRF and 30% HA +70% β-TCP (Osteon II®) bone graft can be used as a regenerative material in treatment of periodontal intrabony defects but being autologous, nonimmune, cost-effective and easily procurable regenerative biomaterial, PRF may be a better option for regeneration.

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