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RESEARCH ARTICLE

COMPARISON OF PRF AND PRF + (HYDROXYAPETITE +BETA TRICALCIUM PHOSPHATE) IN THE TREATMENT OF INFRABONY DEFECT USING CBCT: CLINICORADIOGRAPHIC STUDY

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ABSTRACT

Aim: Aim of the present study is to investigate the clinical and radiological (bone fill) effectiveness of autologous PRF along with the use of alloplastic bone graft material (HA+β TCP) in the treatment of intra bony defects.
Material and methods: A randomized case controlled clinical trial of subjects with 10 intrabony defects which were assigned to group I consisting of intrabony defects treated with PRF alone and group II consisting of 10 intrabony defects on which treatment was done PRF+ bone graft (HA + β TCP) after the initial oral prophylaxis. Evaluation was done at baseline 3 months and 6 months using clinical parameter which included Plaque index (Silness and Loe), Gingival index (Loe and Silness), Probing depth and Relative attachment levels (distance between the most apical portion of the stent and the base of the pocket) and radiographical evaluation was done using a CBCT after 6 months to measure the amount of bone gain obtained.
Results: The statistical result showed significant reduction in plaque and gingival index both in group 1 and 2 and the difference between the group was insignificant. Pocket depth showed statistically significant reduction both in group 1(3mm, 3.10mm at 3 and 6 months) and group 2(4.5mm, 5mm at 3 and 6 months) and group 2 showed significant reduction than group 1(1.5mm and 1.90mm at 3 and 6 months). RAL gain was statistically significant both in group 1(3.10mm and 3.90mm at 3 and 6 months) and group 2(3.70mm, 6.5mm at 3 and 6 months) and group 2 showed significant reduction than group 1(0.60mm, 2.6mm at 3 and 6 months). Statistically significant bone gain was seen in both the groups (1.2mm and 1.32mm at 6 months) but on comparison there was no significant difference between the group.
Conclusion: Therefore, based on the result of this study it is clear that both PRF and combination of PRF+ bone graft (HA + β TCP) are effective in treating infrabony defect and able to improve the clinical and radiographic parameter. However, the improvement in the parameters were better in group 2 compared to group 1, which was due to the better gain in soft tissue in the group 2 than the defect fill.

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INTRODUCTION

Periodontal disease is defined as a complex, multifactorial disease characterized by the loss of connective tissue attachment with destruction of periodontal tissues. The aim of periodontal therapy is to eliminate inflammatory process, prevent the progression of periodontal disease and also to regenerate the lost periodontal tissues (Preeja C; 2014). Periodontal surgical procedures have focused on the elimination of hard and soft tissue defects (i.e., probing depths and osseous defects) by regenerating new attachment (Froum SJ, 1998). Periodontal regeneration is a complex multifactorial process involving biologic events like cell adhesion, migration, proliferation, and differentiation in an orchestrated sequence (William, 1996).

To be considered a regenerative modality, a material or technique must histologically demonstrate that bone, cementum and a functional periodontal ligament (a new attachment apparatus) can be formed on a previously diseased root surface (Zander HA, 1976). Various biomaterials have been used for periodontal tissue regeneration in addition to autogenous and allogenic bone grafts but not a single graft material is considered as gold standard for the treatment of intrabony defects. Platelet-rich fibrin (PRF) may be considered as a second-generation platelet concentrate, using simplified protocol (Dohan *et al*. 2006, Dohan Ehrenfest *et al*. 2009a, b). Carroll *et al*. (2005) in vitro study demonstrated that the viable platelets in PRF released six growth factors like, PDGF, VEGF, TGF, IGF, EGF and βFGF in about the same concentration for the 7-day duration of their study (Choukroun J; 2000) which is prepared from the patient's own blood free of any anticoagulant or other artificial biochemical modifications. It is

advantageous than autogenous graft also because an autograft requires a second surgical site and procedure. Thus, PRF has emerged as one of the promising regenerative materials in the field of periodontics. Calcium phosphate bone grafts are available in different forms viz. hydroxyapatite (HA) and tricalcium phosphate (TCP). Both forms fulfil the requirements for synthetic biomaterials being nontoxic, nonantigenic, noncarcinogenic, stable after sterilization and reasonably inexpensive to fabricate (Ganeles, 1986). HA biomaterials are complex calcium phosphates which resemble bone mineral in their chemical composition $\{Ca_{10}\ (PO4)_6(OH)_2\}$ and has calcium-to-phosphate ratio of 1.67 31. Tricalcium phosphate has a chemical composition of $\{Ca_3\ (PO4)_2\}$ with a calcium-to-phosphate ratio of 1.5 and ismineralogically β-whitlockite (Newman MG; 2010). β-TCP resorbs in faster rate and HA resorbs in a slower rate, this difference in the bioresorption rate may enhance the outcome of periodontal regeneration therapy.Thus the purpose of the present study was to investigate the efficacy of autologous PRF or PRF and HA + β-TCP bone graft with open flap debridement (OFD) in the treatment of intrabony defects.

The present study/case report aims at evaluating the clinically and radiographically the

- Efficacy of PRF in bone regeneration.
- Efficacy of PRF and Bone graft in bone regeneration.
- Comparing the two regenerative techniques – platelet rich fibrin (PRF) and PRF+ bone graft (HA + β TCP) in the treatment of periodontal intrabony defects.

METHODOLOGY

This study was conducted on the subjects visiting the Outpatient Department of A.J. Institute of Dental Sciences, Mangalore. The study sample consisted of 20 sites of infrabony defect in adult subjects with chronic periodontitis (both male and female) and with age ranging from 25-60 years. A brief case history was recorded for all patients taking part in the study. Prior to initiating the study, the patients were informed of the purpose of this randomized clinical trial and were requested to sign an informed consent. Ethical clearance was taken for the study.

Inclusion Criteria

Males and females aged between 25 and 60 years, Chronic periodontitis patients with a minimum of one intrabony defects with clinical probing depth >3mm and <7mm, The presence of infrabony defects on CBCT (2/3 wall confirmed upon surgical exposure), Vital teeth, Teeth with mobility less than grade I(Millers classification), Patients willing to comply with multiple recall schedules.

Exclusion Criteria

- Patients with systemic illness such as diabetes, hypertension, bleeding disorders, epilepsy, or abnormal blood picture, Pregnant/lactating women, Patients on medications known to cause gingival overgrowth or interfere with wound healing, Patients allergic to routine medications prescribed following surgery, Mucogingival problems, Aggressive periodontitis, Smokers, Trauma from occlusion

Selected patients were randomly divided into two experimental groups: Group I was treated open flap debridement (OFD) and PRF was placed (OFD with PRF); and Group II was treated OFD and PRF and HA +β TCP combination was placed. Initial periodontal therapy was done and oral hygiene instructions were given. The patients were reviewed for adequate plaque control. Surgical periodontal therapy was done only when patients achieved a plaque score of zero. Patients were then randomized into the designated study groups. Clinical measurements were noted at baseline, 3 months and 6 months following surgery, radiographical measurement was noted at baseline and at the interval of 6 months. The customised acrylic stent was prepared on the study model for each patient using light cured acrylic to fit every selected patient. Vertical groove was made on the stent at the defect site which guided the placing of the Michigan O with Williams marking probe. This provided reproducibility for probing site.

Clinical Parameters

• Plaque index (Silness and Loe), Gingival index (Loe and Silness), Probing depth, Relative attachment levels (distance between the most apical portion of the stent and the base of the pocket).

Radiographic parameters

• IBD-distance from CEJ to base of the defect
• BD-CEJ to crest of the bone

Protocol for Preparation of PRF

A standard protocol for PRF preparation was followed to obtain proper quantity and quality of the fibrin matrix, leukocytes, platelets, and growth factors. The equipment required for PRF preparation included a R-8C table centrifuge and a blood collection kit consisting of a 24-gauge needle and 10 ml blood collection tubes. A sample of blood was collected from patient without anticoagulant in10 ml tubes which was immediately centrifuged at a rate of 2700 rpm for 12 minutes. After centrifugation, the resultant product consisted of three layers. The topmost layer consisting of acellular PPP (platelet poor plasma), PRF clot in the middle and RBCs at the bottom of the test tube. The fibrin clot obtained after centrifugation was removed from the tube and the attached red blood cells scraped off from it and discarded. PRF was also prepared in the form of a membrane by squeezing out the fluids present in the fibrin clot.

Surgical Procedure

Following the pre-surgical phase, the patients were anesthetized using lignocaine 2% with 1: 100,000 epinephrine. Intraoral asepsis was performed by preprocedural mouth rinse by 10 ml of 0.2% chlorhexidine gluconate solution. Buccal and lingual/palatal sulcular incision was made and mucoperiosteal flaps were reflected. Care was taken to preserve as much of the interproximal soft tissue as possible. After the flap was reflected, the osseous defect was exposed, and thorough surgical debridement of the soft and the hard tissues was carried out using Gracey's area specific curettes. Surgical site was irrigated copiously using normal saline. The defect site was placed with PRF or combination of PRF and HA+βTCP according to the allotted group. The mucoperiosteal flaps were

repositioned and secured using 4-0 interrupted direct loop silk sutures, and the area was protected by non-eugenol dressing.

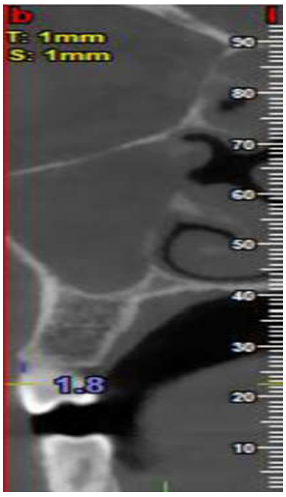
PRF obtained after centrifugation

PRF Layer Seperated

(HA+β TCP) Bone Graft Material

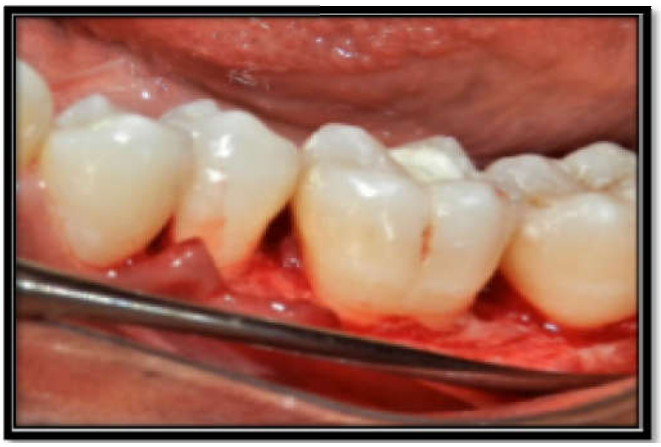
Group 1. Platelet Rich Fibrin Alone

Pre op CBCT IBD measurements

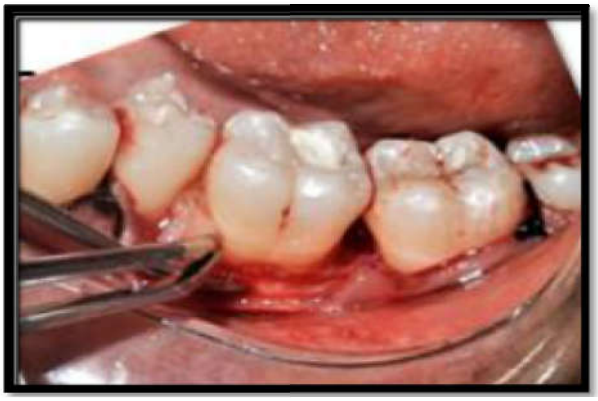


Pre op CBCT BD measurements

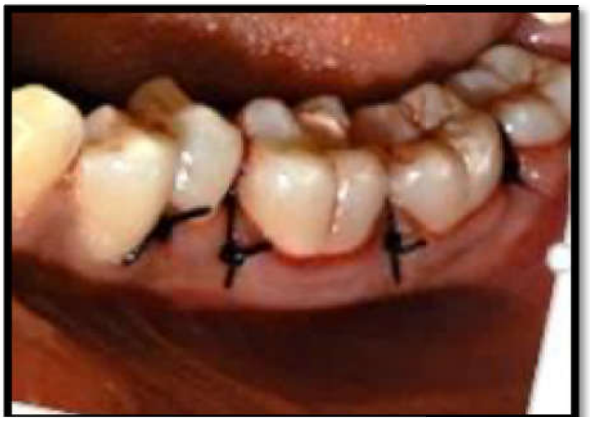
Baseline measurement with michigan 'o' probe and customized stent



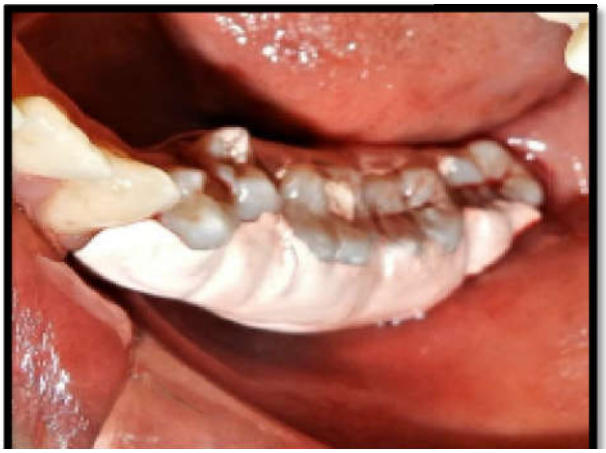
Debridement



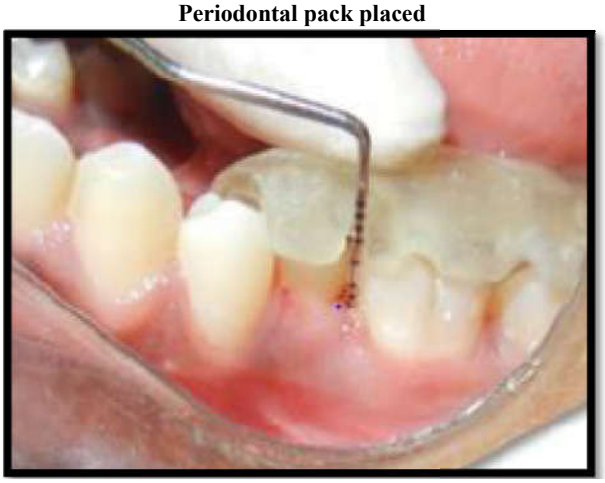
Placement of PRF



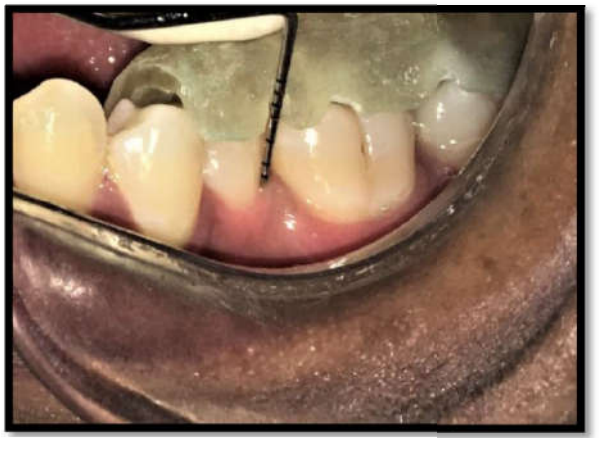
Sutures



Periodontal pack placed

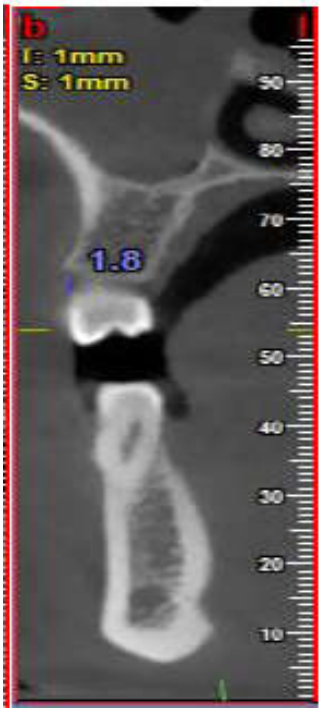


3 Months post op with michigan ‘o’ probe and customized



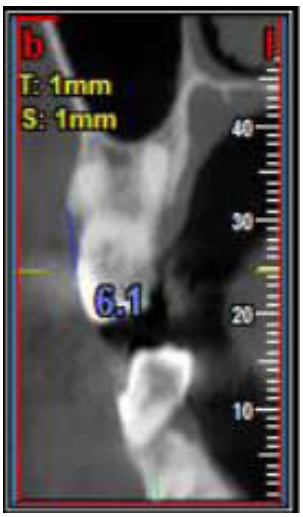
6 Months post op with michigan ‘o’ probe and customized stent

CBCT IBD Measurements after 6 months

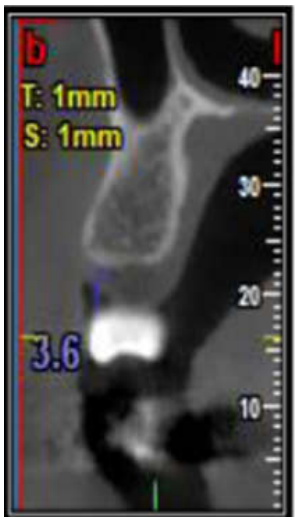


CBCT BD Measurements after 6 months

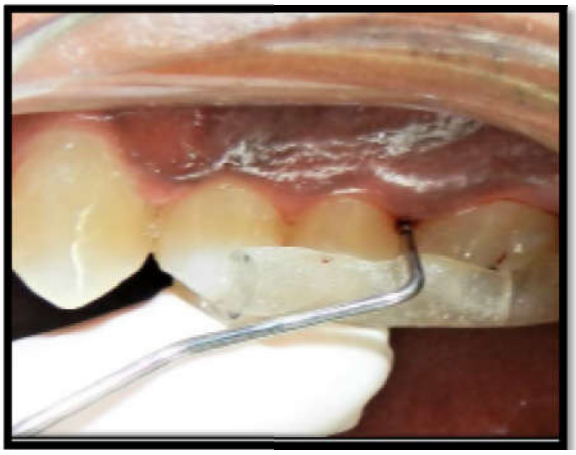
Group 2. Platelet rich fibrin and (HA+B TCP) bone graft



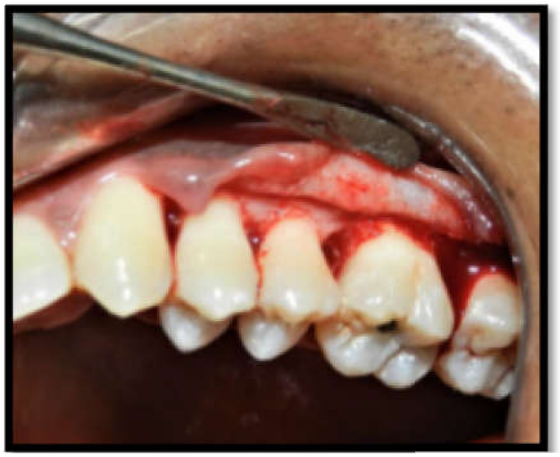
PRE OP CBCT IBD Measurements



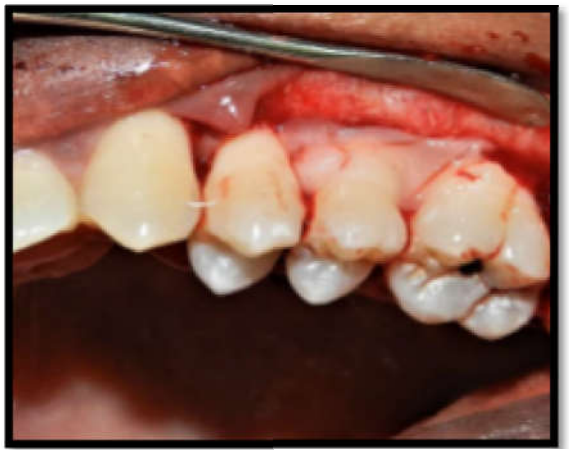
PRE OP CBCT BD Measurements



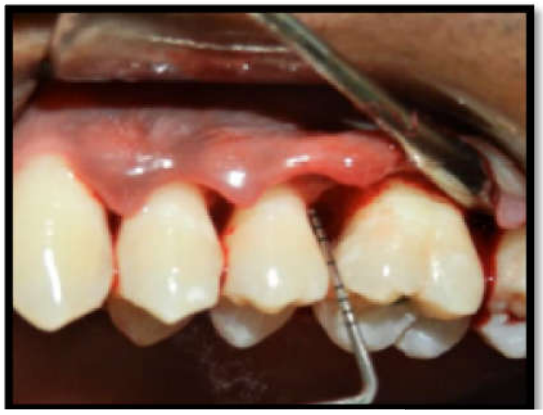
Presurgical probing depth with michigan ‘o’ probe and customized stent



Flap Debridement

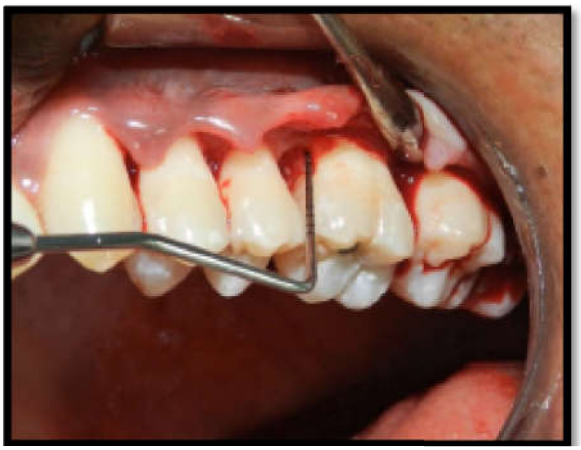


Placement of PRF

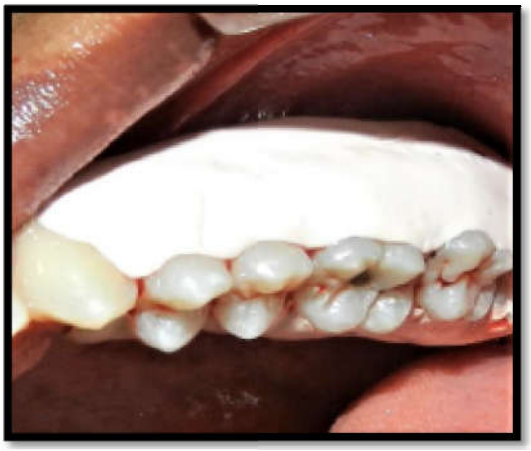


Surgical BD Measurement

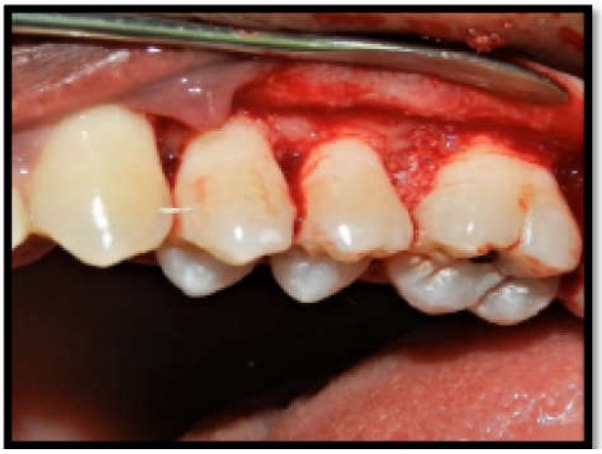
Sutures Placed



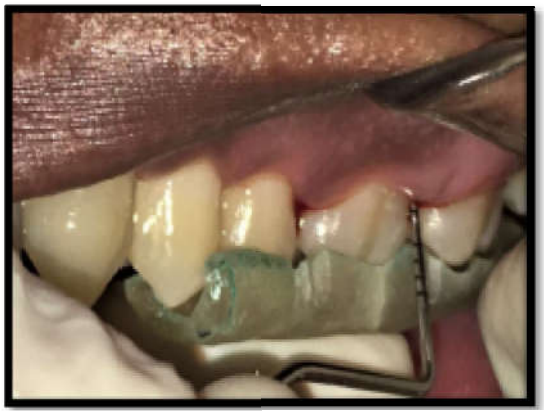
Surgical IBD



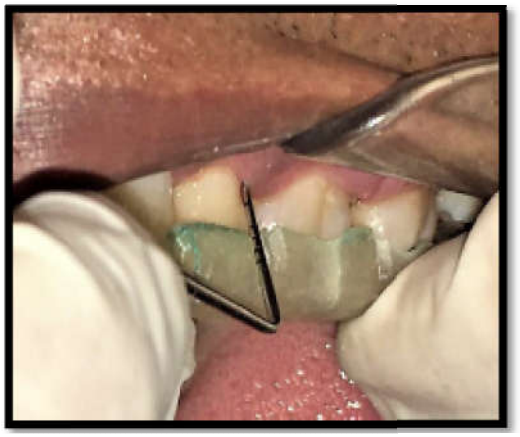
Periodontal Pack Placed



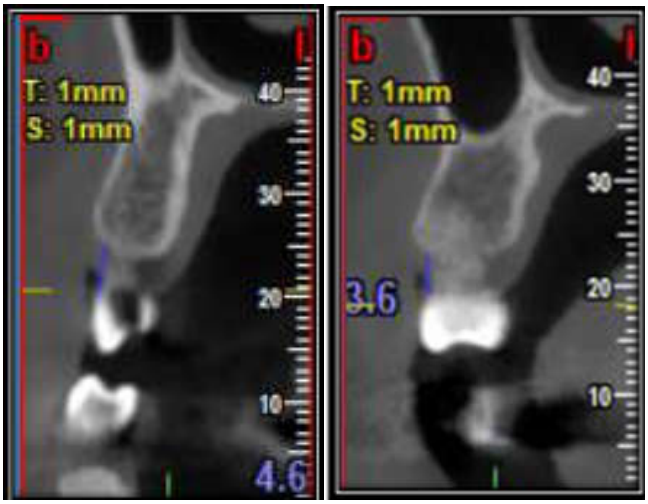
Placement of Bone



3 Months post op with michigan ‘o’ probe and customized stent



6 Months op with michigan 'o' probe and customized stent



CBCT IBD Measurements after 6 months **CBCT BD Measurements after 6 months**

		Values	P value	
Plaque index improvement				
Group 1	At baseline	2.8		
	At 3 months	1.80	<0.05	HS
	At 6 months	1.30		
Group 2	At baseline	2.8		
	At 3 months	1.70	<0.05	HS
	At 6 months	1.30		
Intergroup comparison	At 3 months	.10	>0.05	NS
	At 6 months	0		
Gingival index improvement				
	At baseline	2.8	<0.05	HS
Group 1	At 3 months	1.4		
	At 6 months	1.7		
	At baseline	2.7		
Group 2	At 3 months	1.5	<0.05	HS
	At 6 months	1.7		
Intergroup comparison	At 3 months	.10	>0.05	NS
	At 6 months	0		
Probing depth reduction				
Group 1	At 3 months	3	<0.05	HS
	At 6 months	3.10	<0.05	HS
Group 2	At 3 months	4.5	<0.05	HS
	At 6 months	5	<0.05	HS
Inter group comparison	At 3 months	1.50	<0.05 sig	S
	At 6 months	1.90	<0.05sig	S
Gain in relative attachment level				
Group 1	At 3 months	3.10	<0.05	HS
	At 6 months	3.90	<0.05	HS
	At 3 months	3.70	<0.05	HS
	At 6 months	6.5	<0.05	HS
Intergroup comparison	At 3 months	.60	>0.05	NS
	At 6 months	2.6	<0.05	S
Defect fill				
Group 1	At 6 months	1.22	<0.05	HS
Group 2	At 6 months	1.32	<0.05	HS
Intergroup comparison	At 6 months	.10	>0.05	NS
Crestal bone gain				
Group 1	At 6 months	.14	>0.05	NS
Group 2	At 6 months	.03	>0.05	NS
Intergroup comparison	At 6 months	.11	>0.05	NS

RESULTS

Soft tissue: The mean improvement in Plaque index and gingival index in both group 1 and 2 from the baseline was statistically highly significant (p vlaue <0.05) at 3 months and six months, on comparision between the group it was statistically non significant. Reduction in pocket depth in both group 1 and 2 from the baseline was statistically highly significant (p value <0.05) at 3 months and 6 months. On intergroup comparison there was significant difference in group2. Gain in relative attachment level in both group 1 and group 2 from the baseline was highly significant (p vlaue <0.05) at 3 months and 6 months, on intergroup comparison at 6 months there was significant gain in clinical attachment in group 2

Hard tissue

Defect fill at 6 months was highly significant in both the groups (p vlaue <0.05), on intergroup comparision there was no significant difference between the group. There was no significant gain in the crestal gain in both the group.

DISCUSSION

In this randomised controlled study 20 sites of infrabony defect were selected in which group 1 was treated with PRF and group 2 was treated with PRF and alloplast bone graft (HA + β TCP).

No complications were observed at any of the treated sites. Six-month time frame seems to be the standard for evaluating the success of periodontal regeneration (LekovicV; 2002). Surgical method is considered as the gold standard for evaluating regeneration but in this study, we have used CBCT as the studies have shown that both techniques were equally effective in evaluating regeneration. Along with the high accuracy of CBCT in detection of periodontal bone defects as found in the present study along with its various advantages such as low radiation, rapidity of scan time, and relatively low cost of CBCT comparing to CT, its use is highly advantageous in periodontal practice especially for advanced periodontal disease to more accurately diagnose periodontal disease and its aspects such as amount of bone loss, involvement of furcation, type of defects and their dimension, determine accurately the prognosis of each tooth by allowing 3D analysis of bone around them, and plan for the type of periodontal intervention procedure especially related to regeneration. CBCT may alleviate the need for surgical re-entry to assess bone formation. All these aspect will eventually contribute to significantly improve the quality of periodontal care and thus to improved outcome. Hence, it may not be farfetched to speculate that in near future CBCT may replace the traditional panoramic radiographs and full mouth IOPA for periodontal diagnosis and treatment planning (Banodkar AB; 2015). PRF could improve the periodontal osseous defect healing, as PRF can up regulate phosphorylated extracellular signal regulated protein kinase expression and suppress the osteoclastogenesis by promoting secretion of osteoprotegerin (OPG) in osteoblasts

cultures (Kornman KS; 2000). PRF also demonstrates to stimulate osteogenic differentiation of human dental pulp cells by upregulating OPG and alkaline phosphatase (ALP) expression (Chang IC; 2010). Furthermore, many growth factors are released from PRF as PDGF, TGF and have slower and sustained release up to 7 days (Huang FM;2010) and up to 28 days, which means PRF stimulates its environment for a significant time during remodelling. Moreover, PRF increase cell attachment, proliferation and collagen related protein expression of human osteoblasts (Mazor Z; 2009). Despite of the fact that PRF is a denser and firmer agent than other biological preparations, such as PRP and enamel matrix derivative (EMD), it is still no rigid that its space maintaining ability in periodontal defects is non-ideal. Hence the need to add other bone grafts. PRF increase cell attachment, proliferation and collagen related protein expression of human osteoblasts (Wu CL;2012). PRF also enhances phosphorylated – extracellular signal regulated kinases, OPG and ALP expression which benefits periodontal regeneration by influencing human periodontal ligament fibroblasts (Chang, 2011). To support this, PRF in combination with bone mineral had the ability in increasing the regenerative effects in intrabony defects (Lekovic, 2012). For that reason, we chose alloplast (BONE), hypothesizing that it could enhance the effect of PRF by maintaining the space for tissue regeneration to occur and also provide support for the soft tissue and maintain its position during healing. PRF as defect fillers in combination with alloplast was made due to its ease of manipulation and delivery to the surgical site. The intended role of the PRF in the intrabony defect was to deliver the growth factors in the early phase of healing. Hydroxyapatite is generally considered to be only slightly resorbable, whereas beta-tricalcium phosphate is resorbed by a cellular pathway, leaving newly formed bone tissue.

The different resorption rates of these two constituents provide a diminishing scaffold for bone formation and orderly remodelling over the time. Hydroxyapatite has minimal resorbability and acts as a scaffold for bone ingrowth by providing a fixed structure for calcification to occur. When used as a combination of hydroxyapatite and beta tricalcium phosphate, it has the ability to dissolve, break down, and allow new bone formation and remodel to attain optimal mechanical strength without interference (Bansal S;2009). In the present study plaque index and gingival index scores which was recorded at baseline, 3months and at 6 months following therapy showed significant improvement in the scores. The subjects recruited in this study had varied oral hygiene status which was brought down to minimal Plaque Index scores following scaling and root planing and the baseline values were maintained for all the three groups after the surgery at 3, 6 months. The success of periodontal therapy is based on regular program of recall maintenance and oral hygiene instructions. Periodontal surgical therapy in the absence of an appropriate supportive periodontal therapy will fail eventually. All the subjects were on supragingival plaque maintenance program every month during the follow up period. Notably, patients in our study who failed to comply with the oral hygiene instructions and maintenance schedule, were found to have lesser amount of improvement in the clinical parameters and radiographic assessment. Reduction in PD, IBD and gain in RAL are the major clinical outcomes measured to determine the success of any periodontal treatment. In the present study, a significant reduction in PD and RAL gain were found in both groups when compared with baseline, 3months and 6 months.

There was more PD reduction in group 2 at 3 months (1.5mm > than group 1) and at 6 months (1.90 mm > than group 1) and RAL gain at 3 months (.60mm >than group 1) and at 6 months(2.6mm> than group 1). This supports the significance and advantage of various growth factors present in the PRF which accelerates the soft and hard tissue healing (Dohan et al. 2007). In the meta-analysis done by Shah M et al(2014) showed CAG from 3.03mm to 4.73, reduction in pocket depth in the range of 3.77 to 4.69mm and defect fill in the range of 1.93 to3.20mm which is in accordance with the present study. The present study also reflects the significant amount of bone fill (IBD) both in the group 1 (59%) and the group 2 (61%) with no significant difference between the groups. Joseph VR et al(2012) concluded that greater reduction in PD, more CAL gain and greater intra- bony defect fill at sites treated with autologous platelet rich fibrin as compared to open flap debridement treatment alone which supports the present study. Most of the defects treated in the present study are three walled and two walled defects. One also has to consider that the potential for bone fill may differ depending on the morphology of the angular bone defect.

Most angular defects appear as combinations of one-, two- and three-wall defects and whereas the two- and three-wall component of an angular bone defect may show great potential for bone fill during healing, the one-wall component will rarely demonstrate this type of healing. Besides promoting wound healing, bone growth and maturation, PRF with bone graft have the advantages of graft stabilization, wound healing, hemostasis and improved handling properties (Raja VS; 2008). Study done by Kazuhiro Okuda et al(2005) showed group with PRP and HA. The results compared to baseline, the 12-month indicated that there was significant changes in all clinical parameters (gingival index, bleeding on probing, probing depth, clinical attachment level, and intrabony defect fill; $P < 0.001$), the group with PRP and HA exhibited statistically significant changes compared to the control sites in probing depth reduction: 4.7 ± 1.6), clinical attachment gain: 3.4 ± 1.7 mm and vertical relative attachment gain: 70.3% ± 23.4% which is in accordance with the present study. Pradeep AR et alin the year of 2012 conducted a study in which 90 intrabony defects were treated either with autologous PRF with open flap debridement (OFD) or PRF+HA with OFD or OFD alone. Clinical and radiological parameters such as probing depth (PD), clinical attachment level (CAL), intrabony defect depth and percentage defect fill were recorded at baseline and 9 months postoperatively.

Mean PD reduction was greater in PRF (3.90 ± 1.09 mm) and PRF+HA (4.27 ± 0.98mm) groups than control group (2.97 ± 0.93 mm) while mean CAL gain was also found to be greater in PRF (3.03 ± 1.16 mm) and PRF+HA (3.67 ± 1.03 mm) compared to controls (2.67 ± 1.09 mm). Furthermore, significantly greater percentage of mean bone fill was found in the PRF (56.46 ± 9.26 %) and PRF+HA (63.39 ± 16.52 %) compared to control (15.96 ± 13.91%) which is in accordance with our study. HA when added to PRF increases the regenerative effects observed with PRF in the treatment of human three wall intrabony defects. Bharadwaj k et al(2011) done a study where he used PRF and HA+β TCP to compare with PRF alone found that test group sites showed a significantly higher reduction in pocket depth (4mm) and RAL (5mm) compared to control group sites which is in accordance with our study Bölükbaşı N et alin 2013 had done a Study done which showed that more new bone formation defects filled

with PRF + BCP than PRF alone (Deodhar AK; 1997). The literature includes few studies using alloplast combination graft material of HA and β TCP used with PRF. So, in the present study we have used this combination.

Conclusion

In conclusion, the data from this study suggests, firstly, that treatment of intrabony defects with PRF results in significant improvements of PD, CAL and IBD fill compared with baseline, 3 months and 6 months that HA+β TCP increases the clinical effects observed with PRF in the treatment of human intrabony defects. The use of autologous platelet preparations like PRF allows the clinician to optimize tissue remodelling, wound healing and angiogenesis by the local delivery of growth factors and proteins although HA and TCP provide the required area for the formation of bone due to the nature of the space. According to the results obtained in this case report, it could be concluded that the positive clinical impact of additional application of PRF with biphasic graft material in treatment of periodontal intrabony defect. Use of PRF in the periodontal regeneration procedures would be cost effective and less technique sensitive treatment both for the patients and clinician. However, long term, multicentre randomized, controlled clinical trial will be required to know clinical and radiographical effect over bone regeneration also the long-term results associated with both modalities of therapy, as well as the histological nature of newly formed tissues by either treatment, remains to be elucidated.

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