

Comparative Evaluation of Anorganic Bovine Bone Matrix (ABBM) with or without Platelet Rich Fibrin intreatment of Intrabony Defects:A Randomized Controlled Trial

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Abstract

Background: Risk of alveolar bone loss & tooth loss increases with the presence of intrabony defects. Anoraganic bovine bone mineral (ABBM) is being used in treatment of intrabony defects. Platelet-rich fibrin (PRF) is an autologous concentrate that has an important role in promoting soft and hard tissue healing. This study was done to compare and evaluate the healing of intra-bony defects treated with a combination of ABBM-PRF or ABBM alone.

Method: Twelve patients (24 sites) with intrabony defects were selected and divided randomly in 2 groups. Subjects in Group A (Control group) were treated with ABBM alone & subjects in Group B (test Group) were treated with ABBM + PRF. Clinical parameters such as PPD (Probing pocket depth), CAL (Clinical attachment level), PI (plaque index), GI (gingival index), WHI (Wound healing index), VBL (Vertical Bone Level) & DD (Defect depth) at baseline, 3 and 6 months using acrylic stent.

Result: Significant gain in PPD & CAL (3.92 ± 0.67 to 2.00 ± 0.74) was seen from baseline to 6 months in test group ($p < 0.001$) as compared to control group. Also vertical bone level was increased in test group i.e. from 3.50 ± 0.52 to 3.00 ± 0.43 ($p < 0.001$) post-operative when compared to control group at 6 months.

Conclusion: The addition of PRF to ABBM can augment regeneration and can lead to enhancement of CAL gain.

Keywords: Anaoraganic bovine bone matrix, Blood concentrates, clinical attachment level, growth factors, intrabony defects, platelet rich fibrin, wound healing index, xenograft.

INTRODUCTION

Periodontitis is a chronic inflammatory disease of periodontium causing progressive loss of the soft connective tissue and alveolar bone and causing oxidative stress 1-3. The reduction of the bone height and alteration of the morphological features of bone leads to an array of osseous defects and deformities known as intrabony defects⁴. The concept of regenerative surgery in periodontics comprises of reconstitution of both hard and soft tissues in structure and function⁵. This requires an orchestrated sequence of the biologic events, such as cell migration, adherence, growth and differentiation to have a potential to increase the success and predictability of regenerative procedures⁶. The various treatment modalities aimed to reconstruct the attachment apparatus includes bone grafts, guided tissue regeneration, biomolecular techniques or a combination of any of these approaches⁷. Over the years, various types of graft materials, including autogenic material, have been used to regenerate the attachment apparatus among which xenogeneic materials, anoraganic bovine bone mineral⁷. ABBM is produced by removal of all organic components of bovine bone, whether cancellous or cortical and it is safe to use because as there are no proven systemic or local immune response following its implantation⁸.

Also, in past few years, scientists have concentrated their efforts on biological mediators that have the potential to not only enhance wound healing but also enhance the outcomes of bone replacement grafts⁹ and promote periodontal regeneration by regulating cell proliferation, chemotaxis, and cell differentiation¹⁰. To promote soft and hard tissue healing, various platelet concentrates have been produced¹¹. Platelet – rich fibrin due to its high effectiveness in harvesting leukocytes and platelets. Furthermore, although few clinical studies over the use of PRF for such treatment of intra-bony defects in individuals have been conducted, none have looked into its cumulative impact with ABBM for the management of intra-bony defects¹²⁻¹⁶. Thus, the present randomized controlled study aimed to compare the healing of intra-bony defects treated with a combination of ABBM-PRF or ABBM alone and the current hypothesis being tested was that PRF would augment the regenerative effects of ABBM in human intra-bony defects.

METHODS

The present study was approved by university's ethical committee and was done in agreement with principle guidelines declared in Helsinki experimentation on human subjects. Amongst the patients reporting to the outpatient department of Department of Periodontics and Oral Implantology, Santosh Dental College, Ghaziabad, 12 subjects (24 Sites) were selected for the proposed study based on the specified inclusion & exclusion criteria. Subjects aged 30- 55 years, good oral hygiene with presence of two- or three-wall intra-bony defects with a probing depth (PD) of ≥ 5 mm and an intra-bony component of ≥ 3 mm, as detected on radiographs, good oral hygiene, no intra-bony defects extending into the furcation area were selected for the study and subjects who have undergone periodontal surgery in past 6 months, previous systemic conditions and pregnant females, allergic to local anesthesia, chlorhexidine, antibiotics, and analgesics, habit of smoking and tobacco chewing & compromised immune systems were excluded. Each patient was given detailed verbal and written description of the limitations and was asked to sign a consent form prior to the procedure.

A detailed case history record form was designed for complete patient examination to estimate the prognosis as well as aid in preparing the treatment. A Digital Orthopantomogram and IOPA were done to assess the bone loss pattern in the mouth. At first visit Scaling and root planning was done and patients were recalled after 15 day for surgery. Routine blood investigations were done. Maxillary and Mandibular impressions were made and stent was fabricated on the models. Digital Photographs were taken for each patient at each step. Subjects with acceptable oral hygiene after phase I therapy and requiring surgery with minimum of 2 sites in a single patient in were selected for the study. Patients were assigned to one of the two treatment groups using a computer-generated randomization sequence.

Group-A (ABBM),

Group-B (ABBM+ PRF).

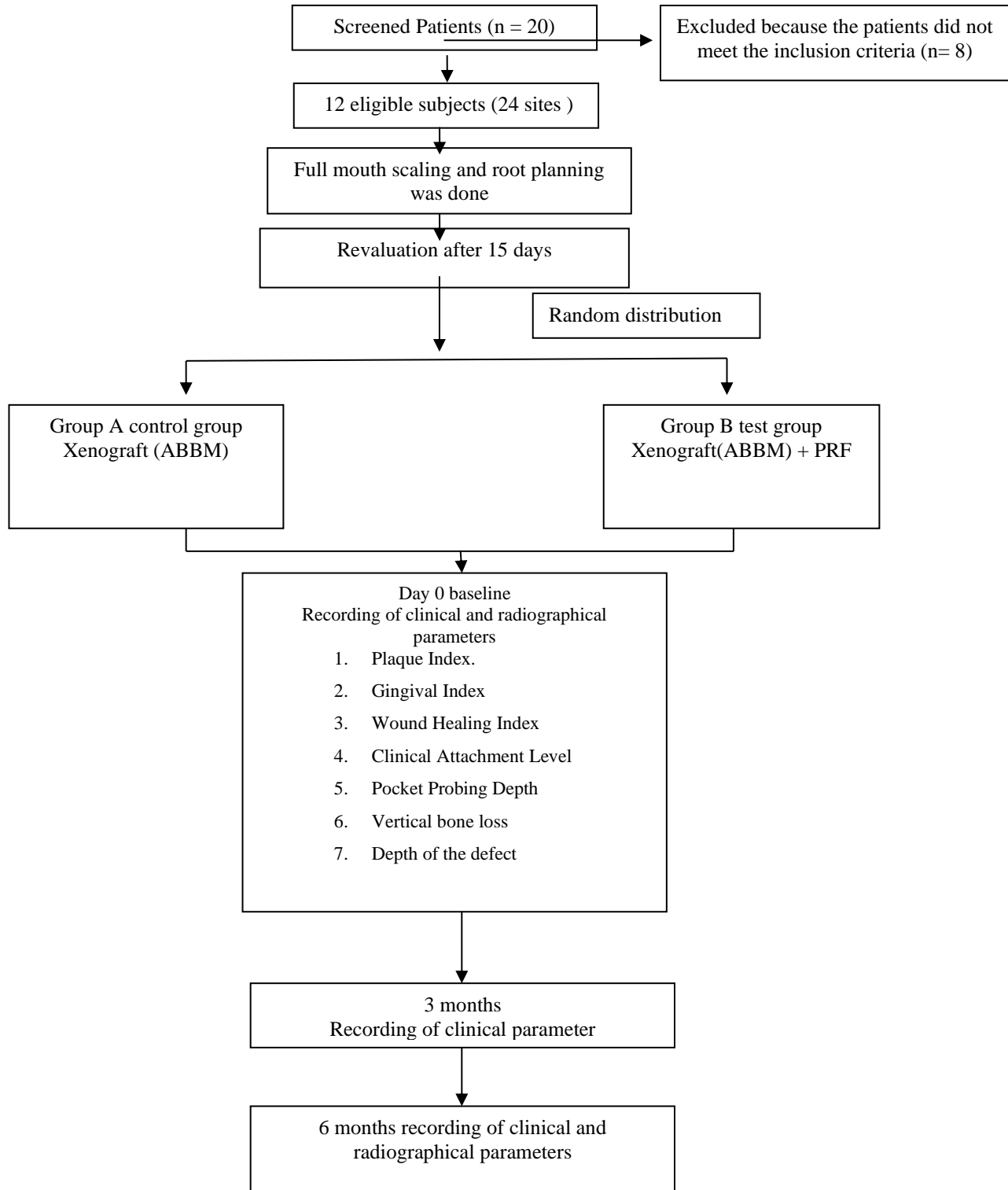
Surgical procedure in each group was : On the day of surgery (Day 0) clinical parameters such as Plaque index, gingival index, pocket probing depth, wound healing index, Clinical attachment level (CAL), and radiographical parameters such as vertical bone loss (VBL) & depth of defect (DD) was recorded preoperatively in both groups, surgical procedure was performed under LA 1:80,000. A full thickness muco-periosteal flap was raised to expose the periodontal structures. The granulation tissue was then cleaned with the help of Gracey Curettes. The surgical site was then irrigated with normal saline in order to remove all the debris from the defect. The area was then isolated and dried. The bone graft was placed at the control site & the bone graft with PRF were placed at the test site.

PRF was prepared according to the procedure described by Choukroun et al¹⁷ by collecting patient's blood from the median cubital vein using a 10 ml syringe and centrifuged at 3000 rpm for 12 min. After centrifugation, the resultant product consists of three layers. The top most layer consisting of acellular PPP (platelet poor plasma), PRF clot in the middle and RBCs at the bottom of the test tube. The PRF clot was removed from the tube with a sterile tweezer (Fig 1). The fibrin clot is separated from the red blood cell fragment, approximately 2mm below the dividing line, using a scissor. Then PRF clot was cut into small pieces with the help of scissors and mixed with the bone graft (Fig 1).

Following the grafting procedure (ABBM alone and ABBM with PRF) (Fig 2), the flap was repositioned and sutured with simple interrupted sutures by using Silk thread (3-0) to obtain the primary closure and the periodontal pack was placed for a week and postoperative instructions were given. Patients were then prescribed antibiotics (Amoxicillin 500mg three times daily for five days) and analgesics (Ibuprofen paracetamol combination). An antimicrobial mouthwash (Chlorhexidine 0.2%)

was advised for one week twice daily. The periodontal dressing and the sutures were removed after 7 days. Oral hygiene instructions were reinforced. Patients were recalled at 7th and 14th day for recording the wound healing index and at 3 & 6 months postoperatively to record the remaining clinical parameters and at 6 months for recording radiographic parameters (Fig 3).

STUDY DESIGN



STATISTICAL ANALYSIS

Statistics were performed by calculating mean and standard deviation for the continuous variables. Software used was SPSS (statistical package for social sciences) version 25.0 and MedCalc software. P-value was taken significant when less than 0.05 ($p < 0.05$) and confidence interval of 95% was taken. Intergroup analysis was performed using unpaired t-test and intragroup analysis was performed using ANOVA.

RESULT:

CLINICAL PARAMETERS

When compared in respect to PI and GI, statistically non significant difference was seen in both the groups at baseline, 3 & 6 months ($p > 0.05$) however on intra-group comparison showed statistically significant difference for PI and GI ($p < 0.001$ using Anova) in both the groups [table 1]

Table 1: Intergroup and intragroup comparison of PI and GI in test and control groups at baseline, 3 and 6 months using unpaired t-test and ANOVA

PI	Mean± SD		p-value
	Group A (control)	Group B (test)	
Baseline	3.33± 0.49	3.17 ± 0.72	0.514
3 months	2.58 ± 0.51	2.25 ± 0.62	0.167
6 months	2.42 ± 0.51	2.25 ± 0.45	0.409
ANOVA(p – value)	<0.001**	< 0.001**	
GI			
Baseline	1.67 ± 0.49	1.67 ± 0.49	1.000
3 months	0.67 ± 0.49	0.83 ± 0.72	0.514
6 months	0.33 ± 0.49	0.33 ± 0.49	1.000
ANOVA(p – value)	< 0.001**	< 0.001**	

PI – Plaque Index ; GI – Gingival Index ; SD – Standard Deviation

*Statistically significant at a $P < 0.05$ (unpaired t-test)

** Statistically significant at a $p < 0.001$ (ANOVA test)

Table 2: Intergroup and intragroup comparison of PPD and CAL in test and control groups at baseline, 3 and 6 months using unpaired t-test and ANOVA

PPD	Mean± SD		p-value
	Group A (control)	Group B (test)	
Baseline	6.83±0.72	7.08±0.67	0.387

3 months	6.17±0.58	5.75±1.06	0.243
6 months	5.83± 0.58	5.50± 1.00	0.328
ANOVA(p – value)	<0.001**	< 0.001**	
CAL			
Baseline	3.92± 0.67	3.92 ±0.67	1.000
3 months	3.33 ± 0.65	2.08 ± 0.79	0.001*
6 months	3.17± 0.58	2.00 ± 0.74	0.001*
ANOVA(p – value)	< 0.001**	< 0.001**	

PPD – Probing Pocket Depth ; CAL – Clinical attachment Level ; SD – Standard Deviation

*Statistically significant at a P < 0.05 (unpaired t-test)

** Statistically significant at a p <0.001 (ANOVA test)

Table 3: Intergroup and intragroup comparison of VBL and DD in test and control groups at baseline & 6months using unpaired t-test and ANOVA

VBL	Mean± SD		p-value
	Group A (control)	Group B (test)	
Baseline	7.08 ± 1.31	7.08 ± 1.31	1.000
6 months	3.50 ± 0.52	3.00 ±0.43	0.018*
ANOVA(p – value)	<0.001**	< 0.001**	
DD			
Baseline	4.50 ± 0.52	4.00 ± 0.60	0.141
6 months	1.83 ± 0.58	1.67 ± 0.49	0.455
ANOVA(p – value)	< 0.001**	< 0.001**	

VBL – Vertical Bone Level ; DD – Defect Depth; SD – Standard Deviation

*Statistically significant at a P < 0.05 (unpaired t-test)

** Statistically significant at a p <0.001 (ANOVA test)

Table 4: Intergroup and intragroup comparison of WHI in test and control groups at baseline,3 and 6months using unpaired t-test and ANOVA

WHI	Mean± SD		p-value
	Group A (control)	Group B (test)	
7 days postoperative	1.58 ± 0.51	1.17± 0.39	0.056
14 days postoperative	1.42 ± 0.51	0.42± 0.51	0.001*
ANOVA(p – value)	0.166	< 0.001**	

WHI – Wound Healing Index; SD – Standard Deviation

*Statistically significant at a P < 0.05 (unpaired t-test)

** Statistically significant at a p <0.001 (ANOVA test)

The difference in mean value of wound healing index when compared between group A and group B from 7th to 14th day was found to be statistically significant (p <0.001) [table 4]

Probing Pocket Depth decreased significantly in group A (6.83mm to 5.83mm) & group B (7.08 mm to 5.50mm) when compared at baseline to 6 months for both groups whereas on intergroup comparison there was no statistically significant difference between was seen between the two groups ((p >0.05) [table 2]

Statistically significant CAL gain was seen in both the groups i.e Group A(3.92mm to 3.17mm) and Group B(3.92mm to 2.00mm) & the mean difference was found to be statistically significant in intergroup comparison from base line to 6 months (p<0.001).[table 2]

RADIOGRAPHICAL PARAMETERS

On analysis of hard tissue i.e alveolar bone, both the treatment groups showed significant improvements compared to baseline in terms of vertical bone level at 6 months (p < 0.05). (table 3) Whereas the defect fill was seen in intragroup comparison from baseline to 6 months, but no statistically significant difference was seen on intergroup comparison (p > 0.05).[table 3]

DISCUSSION

Intrabony defect is not just any osseous defect with the base on a periodontal pocket apical to the alveolar crest; it is a distinct osseous defect with specified form. When bone graft material is implanted and maintained in the periodontal defects, it has a varying capacity to stimulate coordinated growth of bone, cementum & PDL. Anorganic bovine bone material is biocompatible and has high osteoconductive potential with limited resorptive potential. The use of a mixing blood derivative in conjunction with a bone graft improves the predictability of the treatment and the amount of bone healing Choukroun et al.2000 in France were the first to develop PRF for use in oral and maxillofacial surgery¹⁷. For seven days, PRF release six growth factors at the same concentration: PDGF, VEGF, TGF, IGF, and EGF (Throat et al)¹². Since not many studies have been done, so this study evaluated and compared the clinical and radiographical efficacy of anorganic bovine bone mineral (ABBM) with or without platelet rich fibrin (PRF) in treatment of intrabony defects. In the present study, when an intra-group comparison was made it was found that the clinical parameters such as PI, GI, WHI, PPD, CAL and Radiographical parameters such as VBL and DD were reduced significantly in both the test and control groups when compared from baseline to 3 and 6 months. Furthermore, according to intergroup analysis, wound healing index score was decreased, there was reduction of probing pocket depth and vertical bone level, and the CAL gain was found to be statistically significant in test group when compared to control group, however, other parameters such as PI, GI and DD did not show significant differences on intergroup comparisons. As there was no significant differences in the values of the parameters on inter group comparison at the baseline, thus preventing the selection bias for both the procedures at the beginning of the study. Similarly in the study by Bhatia G et al (2018)¹⁸ the author performed

a comparative evaluation of porous hydroxyapatite bone graft with or without platelet rich plasma and found no statistically significant differences in the plaque and gingival Index scores in intergroup comparison. In another study by Mathur A et al (2015)¹⁹ inter group comparison reduction in PI and GI score was not significant when compared between test and control group from baseline to six months. The wound healing index which was followed in the current study was suggested by Huanget al in 2005²⁰. In the current study statistically significant reduction was seen in WHI scores from 7 to 14 days post operative. In the test group hence indicating the better early healing which can be attributed to the growth factors contained in PRF. In terms of probing pocket depth it was found that there was statistically significant decrease in the mean probing depths from baseline to 6 months in the test group. These findings are in concordance with the study done by Richardson CR 1999²¹, Carmago et al 2000²², Sculean A et al 2003²³, Tonetti MS et al 2004²⁴, Parimala M et al in 2010²⁵, Lekovic V et al in 2011²⁶, Mathur A et al 2015¹⁹ & Bhatia G et al 2018¹⁸. Conversely, as per studies done by Dori et al 2009²⁷ & Sezgin Y et al in 2017²⁸ and no statistically significant differences were appreciated between the two treatment groups. In the study, statistically significant CAL gain was seen in the test group when compared to be control group at 6 months post surgery. These significant findings of gain in attachment levels can be attributed to the use of PRF as also seen in studies conducted by Sezgin Y et al in 2017²⁸. Similar results were seen in the studies done by Carmago et al 2005²⁹, Hanna et al 2004³⁰, Parimala M et al in 2010⁽²⁵⁾ & Chandradas N et al 2016³¹. In terms of radiographic parameters (VBL & DD) in this study it was found that statistically significant decrease was appreciated in VBL. Same results for the VBL at 6 months postoperatively were also found in the studies done by Richardson CR 1999³², Sezgin Y et al in 2017²⁸ and Linares A in 2006³². Conversely, in the study by Ilgenli T et al in 2007³³ it was seen that when the defect was treated with DFDBA + PRP did not show statistically significant reduction in the depth of defect. In the test group when compared to the control group whereas there were no statistically significant changes in DD on inter group comparison. Many studies have examined¹²⁻¹⁴, the additive effects of autologous PRF in the treatment of intrabony defects and have arrived at the conclusion that PRF improves both Clinical and radiographic parameters. This study confirms similar findings where in statistically significant differences were observed in PPD, CAL, WHI & VBL in the test group (ABBM + PRF) when compared to control group (ABBM alone). ABBM being an extensively researched material, has successfully proved beneficial in the treatment of intra bony defects with a positive role in reducing PPD, improving attachment levels and increasing bone levels in periodontal defects^{22,26,3}. Choukroun's method does not require anticoagulants or bovine thrombin, but does require a Process of PC-02 table centrifuge for PRF preparation and a collection kit from Nice, France; hence, PRF polymerizes naturally and slowly during centrifugation. The first and foremost drawback of this study is a small evaluation period of 6 months, for better results and conclusion a extended follow up should be done. Furthermore, study limitations could include a lack of histological testing to assess PRF's regeneration capacity, a lack of evaluation of patient-reported results (using visual analog scale to test pain sensitivity etc)²⁸, not using the GTR membrane and smaller sample size.

CONCLUSION

On the basis of the results, we can conclude that while both treatment modalities i.e, ABBM in combination with PRF and ABBM alone have shown promising results individually in the treatment of intrabony periodontal defects, however on comparison between both test group and Control group it was seen that there was statistically significant reduction in probing pocket depth, vertical bone level, wound healing index and gain in Clinical attachment level in test group than control group. PRF with ABBM was safe to use without causing any immunogenic or antigenic reaction in any of the treated patients. Both of the group showed the potential of enhancing the periodontal regeneration with the PRF group showing better benefits. However, future studies with larger sample size and longer duration should be undertaken to collaborate the result of the current study.

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