A COMPARATIVE EVALUATION OF AUTOLOGUS GRAFT V/S ALLOPLAST FOR ALVEOLAR RIDGE PRESERVATION IN EXTRACTION SOCKET- A CLINICO- RADIOGRAPHIC STUDY

Dissertation

Submitted to

BABU BANARASI DAS UNIVERSITY, LUCKNOW, UTTAR PRADESH

In the partial fulfilment of the requirements for the Degree

Of

MASTER OF DENTAL SURGERY

In

PERIODONTICS

By

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BATCH: 2014-2017

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled "A COMPARATIVE EVALUATION OF AUTOLOGUS BONE GRAFT V/S ALLOPLAST FOR ALVEOLAR RIDGE PRESERVATION IN EXTRACTION SOCKET- A CLINICO-RADIOGRAPHIC STUDY" is a bonafide and genuine research work carried out by me under the guidance of ***Dr. VANDANA A PANT***, Professor, Department of Periodontics, Babu Banarasi Das College Of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

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ACKNOWLEDGEMENT

"If you have knowledge, let others light their candles in it. A candle loses nothing by lighting another candle"

I owe my deepest gratitude to my guide Dr. Vandana A. Pant, Professor, Department of Periodontics, Babu Banarasi Das College of Dental Sciences, Lucknow, who patiently provided the vision, advice and encouragement necessary for me to proceed through and complete my dissertation. Her vast knowledge and ability to achieve excellence has proved to be very valuable throughout. I shall always remain greatly thankful for the scholarly guidance provided by her.

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I am highly thankful to my H.O.D Dr. Vivek Govila, Principal Professor, Department of Periodontics, Babu Banarasi Das College of Dental Sciences who patiently provided the vision and providing me a opportunity and space for completion of my dissertation.

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Dr. Kumar Shantanu

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DEDICATED

TO

MY FAMILY

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ABBREVIATIONS

ARP	Alveolar Ridge Preservation
β-TCP	Beta -Tricalcium Phosphate
PRF	Platelet Rich Fibrin
PRP	Platelet Rich Plasma
PRGF	Plasma Rich growth Factor
CT	Computed Tomography
CBCT	Cone Beam Computed Tomography
3D	Three Dimensonal
PDGF	Platelet Derived Growth Factor
IGF	Insulin Like Growth Factor
VEGF	Vascular Endothelial Growth Factor
VCR	Volumetric Computed Radiography
ALP	Alkaline Phosphatase
PRFM	Platelet Rich Fibrin Matrix
DFDBA	Demineralized Freez Dried Bone Allograft
RVG	Radiovisiography
CEJ	Cemento Enamel Junction

IL-1β	Interleukin - 1 Beta
TNF-α	Tumor Necrosis Factor-Alpha
IL-2	Interleukin - 2
IL-4	Interleukin - 4
IL-6	Interleukin - 6

Abstract

ABSTRACT

Dental caries, periodontal disease, and trauma if its remains untreated lead to progressive loss of tooth structure, supporting periodontium, and alveolar bone dimenssion and height. Alveolar ridge preservation therefore becomes important for any future restoration.

Total of 20 patients irrespective of gender, suffering from chronic periodontitis with grade-III mobility or having grossly decayed non restorable tooth were selected from the O.P.D of the department. Radiographic analysis by CBCT was done at base line and 4 months after the procedure for accurate measurement.

The patients were divided into 2 groups after the diagnosis and out of 20 extraction sites, 10 sites were selected for PRF alone (Group A) and 10 sites with combination of PRF and Beta Tri-Calcium Phosphate(Group B).

Group A patients got their tooth extracted atraumaticaly and PRF was grafted inside the socket, sutures were given and same procedure was also done for Group B (combination of PRF and Beta Tri-Calcium Phosphate) patients. CBCT was done at base line and after 4 months for comparing the changes in vertical bone height and bone width at 0mm, 1mm and 5mm from the crest.

Both the groups showed significant gain in alveolar ridge preservation but the Combination of PRF with Beta Tri-Calcium phosphate was significantly more effective over PRF alone. Although there was resoption of ridge width buccolingually in both the groups but it was significantly much less with group B i.e combination of PRF and Beta Tri-Calcium Phosphate.

Key Words- PRF, Beta Tri-Calcium phosphate and Alveolar ridge preservation.

Introduction

INTRODUCTION

Dental caries, periodontal disease, and trauma if its remains untreated lead to progressive loss of tooth structure, supporting periodontium, and alveolar bone. Such types of teeth with hopeless prognosis are indicated for extractions.[1]

Most extractions are done with no regard for maintaining the alvolar ridge. Post extractions the subsequent healing of the socket commonly result in osseous deformities of the alveolr ridge, including reduced vertical height and reduced horizontal width of the residual alveolar ridge.[2]

The main problem for the clinicians is how to preserve a ridge dimensions or bone volume during or after extractions, leading to improper base for placement of dental implants and conventional prosthesis.[3]

The dental literature describes loss of bone volume after dental extractions of up to 50% within 6 months. Approximately 0.34 to 7.7 mm of horizontal ridge reduction and 0.2 to 3.25 mm of vertical ridge reduction occurs in the 6 to 12 months period following extraction[4,5], primarily in the initial 3 months. While the size of the residual ridge is reduced more rapidly in the first 6 months, bone resorption activity continues at a slower rate throughout life.[4]

Dental implants for single-tooth replacements have evolved into a viable prosthodontic alternative to conventional fixed bridgework, resin bonded restorations or removable partial dentures. Long-term studies have reported excellent implant survival rates with satisfactory esthetic out- come and long-term functional stability when applied for tooth replacements with alveolar ridge preservation after extraction.[5]

With the aim of minimizing the need for future tissue augmentation, several authors have proposed techniques to preserve the anatomy of the alveolar ridge after tooth extraction. These procedures have collectively been termed alveolar ridge preservation (ARP) or socket preservation. Several different ARP techniques exist, most of which include the use of a graft materials which can be autogenic, allogeneic, or synthetic bone graft material, or a combination of these products.[6,7]

Beta-tricalcium phosphate (β-TCP), a synthetic alloplastic material, has been used for bone regeneration in a variety of surgical procedures with satisfactory clinical and histologic results in both animal models and human trials. β-TCP is a resorbable bone graft, osteoconductive and biocompatible in nature.[8] It is commercially available as Resorbable Tissue Replacement (RTR) cone (Septodont, Saint-Maur-des-Fosses, France) for reconstruction of bone defects in maxillofacial and dental surgeries.[9]

Choukroun, first developed platelet-rich fibrin (PRF) which is a second-generation platelet concentrate.[11] Since then, it has been used for the management of intrabony defects, socket preservation procedure,sinus lift techniques for implant placement and coverage of recession defects in the form of a membrane.PRF is a viable and biocompatible autologous biologic material that can be used alone to maintain ridge dimension during preservation procedures while at the same time stimulating rapid osseous fill of the socket.[12]

PRF membrane stimulates its environment for a substantial period of remodeling and has a very significant slow unremitting release of critical growth factors for at least 7

days up to 28 days. The properties of this natural fibrin biomaterial thus offer a great potential for wound healing.[13] PRF is cost effective, biocompatible and significantly same as β-TCP, when we use alone.

Routine dental x-rays are two-dimensional; they only show the location of the teeth and the height of the bone and they cannot depict the thickness of the jawbone. So now a days Cone-beam computerized tomography (CBCT) has been used.

CBCT appears to offer an effective, non-invasive and relatively low radiation technique for assessment of dimensional changes in the alveolar ridge.

As per the literature search, there is no study where the PRF as an autologous graft in combination with β-TCP, an alloplastic graft and PRF alone has been compared for the outcome of alveolar ridge preservation in extraction socket using 3D imaging i.e (CBCT) in human beings.

Aims & Objectives

AIM:-

This study was taken up with the aim to compare the effectiveness in clinical outcome of combination of Platelet Rich Fibrin (PRF) with β- Tricalcium phosphate as graft material and PRF alone for alveolar ridge preservation in extraction socket. CBCT was done for all the cases to get near accurate measurements.

OBJECTIVES:-

To compare the changes in the alveolar bone dimensions i.e vertical height and the buccolingual width of the extracted socket grafted with PRF alone and combination of PRF and β- Tricalcium phosphate with the help of CBCT at baseline and after four months.

Review of Literature

Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J et al (2005)[14] described the conceptual and technical evolution from fibrin glues to platelet concentrates. Retrospective analysis was carried out for the understanding of fibrin technologies and the evaluation of the biochemical properties of 3 generations of surgical additives, respectively fibrin adhesives, concentrated platelet-rich plasma (cPRP) and PRF. Indeed, the 3-dimensional fibrin architecture is deeply dependent on artificial clinical polymerization processes, such as massive bovine thrombin addition. According to them, the slow polymerization during PRF preparation seems to generate a fibrin network very similar to the natural clot. Such a network leads to a more efficient cell migration and proliferation.

Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, et al (2005)[15] investigated the platelet-associated biochemical property of PRF. During PRF processing by centrifugation, platelets are activated and their massive degranulation implies a very significant cytokine release. To quantify PDGF-BB, TGFb-1, and IGF-I within PPP (platelet-poor plasma) supernatant and PRF clot exudates serum. Slow fibrin polymerization during PRF processing leads to the intrinsic incorporation of platelet cytokines and glycanic chains in the fibrin meshes. They hypothesized that PRF would be able to progressively release cytokines during fibrin matrix remodeling; such a mechanism might explain the clinically observed healing properties of PRF.

Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J et al (2005)[16] investigated the immune features of PRF. During PRF processing, leucocytes could also secrete cytokines in reaction to the hemostatic and inflammatory phenomena artificially induced in the centrifuged tube. Authors therefore undertook to quantify 5

significant cell mediators within platelet poor plasma supernatant and PRF clot exudate serum: 3 proinflammatory cytokines (IL-1β, IL-6, and TNF-α), an antiinflammatory cytokine (IL-4), and a key growth promoter of angiogenesis (VEGF). Data was correlated with that obtained in plasma (nonactivated blood) and in sera (activated blood). These initial analyses revealed that PRF could be an immune regulation node with inflammation retrocontrol abilities. This concept could explain the reduction of postoperative infections when PRF is used as surgical additive.

Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL et al (2005)[17] investigated previously evaluated biology of PRF with the first established clinical results, to determine the potential fields of application for this biomaterial. The reasoning is structured around 4 fundamental events of cicatrization, namely, angiogenesis, immune control, circulating stem cells trapping, and wound-covering epithelialization. All of the known clinical applications of PRF highlight an accelerated tissue cicatrization due to the development of effective neovascularization, accelerated wound closing with fast cicatricial tissue remodelling, and nearly total absence of infectious events. This initial research therefore makes it possible to plan several future PRF applications, including plastic and bone surgery, provided that the real effects are evaluated both impartially and rigorously.

Casati MZ, Gonçalves PF, Pimentel SP, da Rocha, Filho G, Nociti FH Jr et al (2006)[18] conducted a study to evaluate the influence of platelet rich plasma (PRP) on bone regeneration in dehiscence-type bone defects around dental implants. Ten male adult mongrel dogs were used. Three months after teeth extractions, an osteotome for implantation and a buccal dehiscence defect were prepared on both sides of the jaws.

Two dental implants with machined surfaces were placed on each implant site of the mandible. Dehiscences were randomly assigned to the following groups: (1) test (PRP) and (2) control. After 3 months animals were sacrificed; implants and adjacent hard tissues were processed for undecalcified sections. Bone-to-implant contact, bone density and new bone area in a zone lateral to the implant, corresponding to bone defects, were obtained and measured. Inter group analysis (paired Student's t-test, a = 5%) demonstrated no statistically significant differences for any of the parameters when PRP was used ($P > 0.05$). Within the limits of the present study, it was concluded that platelet-rich plasma alone did not enhance bone regeneration for peri-implant defects.

Brkovic BM, Prasad HS, Konandreas G, Milan R, Antunovic D, Sándor GK et al (2007)[19] conducted study on preservation of a maxillary extraction socket using beta-tricalcium phosphate with type I collagen without the use of barrier membranes or flap surgery. Clinical examination revealed solid new bone formation 9 months after the procedure. At the time of implant placement, histomorphometric analysis of the biopsied bone showed that it contained 62.6% mineralized bone, 21.1% bone marrow and 16.3% residual beta -TCP graft. The healed bone was able to support subsequent dental implant placement and loading.

Dohan Ehrenfest DM, Diss A, Odin G, Doglioli P, Hippolyte MP, Charrier JB. (2009)[20] conducted study was to analyze the effects of Choukroun's PRF, a leucocyte and platelet concentrate clinically usable as fibrin membrane or clot, on human primary cultures of gingival fibroblasts, dermal prekeratinocytes, preadipocytes, and maxillofacial osteoblasts. For the proliferation study, these cells were cultivated with

or without a PRF membrane originating from the same donor as for the cells. For osteoblasts and fibroblasts, dose-dependent effect was assessed (using 2 membranes). Cell counts and cytotoxicity tests were performed at 3, 7, 14, and 21 days, and even 28 days for osteoblasts. Authors concluded that PRF induced a strong differentiation in the osteoblasts and scanning electron microscopy, revealed a starting mineralization process in the PRF membrane itself after 14 days. Moreover, PRF leucocytes seemed to proliferate and interact with osteoblasts.

He L, Lin Y, Hu X, Zhang Y, Wu H (2009)[21] conducted a study to evaluated the effect of biologic characteristics of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) on proliferation and differentiation of rat osteoblasts. Blood samples were collected from 14 healthy volunteers (7 male) with a mean age of 23.2 ± 2.24 years. PRP and PRF were prepared with standard protocols. The exudates of PRP and PRF were collected at the time points of 1, 7, 14, 21, and 28 days. The levels of platelet-derived growth factor AB (PDGF-AB) and transforming growth factor β1 (TGF-β1) were quantified in PRP and PRF. Then the exudates of PRP and PRF were used to culture rat calvaria osteoblasts. The biologic characteristics of osteoblasts were analyzed in vitro for 14 days. PRP released the highest amounts of TGF-β1 and PDGF-AB at the first day, followed by significantly decreased release at later time points. PRF released the highest amount of TGF-β1 at day 14 and the highest amount of PDGF-AB at day 7. PRF release autologous growth factors gradually and expressed stronger and more durable effect on proliferation and differentiation of rat osteoblasts with increased expression of ALP and induction of mineralization than PRP in vitro.

Mazor Z, Horowitz RA, Del Corso M, Prasad HS, Rohrer MD, Dohan Ehrenfest DM (2009)[22] conducted a study to assessed the relevance of PRF clots and membranes as the sole filling material during a lateral sinus lift with immediate implantation using radiologic and histologic analyses in a case series. Twenty-five sinus elevations with simultaneous implantation were performed on 20 patients with Choukroun's PRF as the sole filling biomaterial. For each patient, a presurgical exam and a 6-month post-surgical radiologic exam were performed with a panoramic x-ray and three-dimensional volumetric computed radiography (VCR) to evaluate the subsinus residual bone height and the final bone gain around the implants. From a radiologic and histologic point of view at 6 months after surgery, the use of PRF as the sole filling material stabilized a high volume of natural regenerated bone in the subsinus cavity up to the tip of the implants.

B.I Simon, A.L Zatcoff, J.J.W Kong, and S.M O'Connell (2009)[23] conducted a study the healing potential of platelet growth factors in ridge preservation procedures. A canine study was performed to determine if extraction sites treated with platelet-rich fibrin matrix (PRFM) exhibit enhanced healing compared to sites treated with non-viable materials. Four dog's extraction sockets were treated individually with PRFM, PRFM and membrane, DFDBA and membrane, PRFM and DFDBA, and untreated control. Treatment sequencing permitted clinical and histologic evaluation of healing at 10 days, 2, 3, 6 and 12 weeks. Healing was more rapid in the PRFM and PRFM and membrane sites. By 3 weeks those sockets had osseous fill. Sites containing DFDBA had little new bone at 6 weeks. By 12 weeks those sockets had osseous fill but DFDBA particles were still noted in coronal areas. Study concludes

PRFM alone may be the best graft for ridge preservation procedures with advantages like faster healing, and elimination of disadvantages involved in using barrier membranes.

A. Simonpieri, J. Choukroun, M. Del Corso, G. Sammartino, and D. M. Dohan Ehrenfest (2011)[24] conducted a study in their 6-year follow-up study, were able to demonstrate that using PRF as a sole grafting agent was a viable long-term option in sinus augmentation procedures. To assess the relevance of simultaneous sinus lift implantation with leukocyte and platelet rich fibrin. This autologus and inexpensive material can be considered as optimized blood clot and this L-PRF matrix seems a relevant biomaterial for natural bone regeneration.

Jiing-Huei Zhao, Chung-Hung Tsai, Yu-Chao Chang (2011)[25] published a case report on the clinical and histologic characteristics of a patient who underwent a tooth extraction which was filled with PRF. At the time of implant insertion, the socket was completely filled by a hard material, which on probing exhibited the consistency of bone. A histological examination of the core taken from the socket revealed new bone formation. The clinical and histological findings suggest that filling a fresh extraction socket with PRF provides a viable therapeutic alternative for implant site preparation.

Horowitz R, Holtzclaw D, Rosen PS (2012)[26] conducted a review on alveolar ridge preservation following tooth extraction. The evidence was obtained by search of Entrez PubMed and manual search of The International Journal of Oral and Maxillofacial Implants, The International Journal of Periodontics & Restorative Dentistry, Clinical Oral Implant Research, The Journal of Periodontology and The

Journal of Clinical Periodontology. Reviewed literature supporting ridge preservation techniques as a whole. Multiple studies demonstrated less ridge resorption occurring when alveolar ridge preservation procedures were used versus the placement of no graft material in fresh alveolar sockets.

Barone A, Ricci M, Tonelli P, Santini S, Covani U (2012)[27] conducted a study to evaluated and compared changes of hard and soft tissues in post-extraction sockets which received a ridge preservation procedure, with post-extraction sockets which had healed naturally. The control sites received silk sutures to stabilize the clot without any grafting material. The test sites were grafted with corticocancellous porcine bone and a collagen membrane. Clinical parameters were evaluated at baseline and after 4 months at implant placement: vertical bone changes, horizontal bone changes and width of keratinized gingiva. This study clearly points out that an alveolar ridge preservation technique was able to limit the contour changes after tooth extraction. Finally, the grafted sites allowed the placement of longer and wider implants when compared to implants inserted in non-grafted sites.

Chappuis V, Engel O, Reyes M, Shahim K, Nolte LP, Buser D (2013)[28] conducted a study to showed that dimensional alterations of the facial bone wall following tooth extractions in the esthetic zone had a profound effect on treatment outcomes. This prospective study in 39 patients was the first to investigate three-dimensional (3D) alterations of facial bone during the initial 8 weeks following flapless tooth extraction. A novel 3D analysis was carried out, based on 2 consecutive cone beam computed tomographies (CBCTs). A risk zone for significant bone resorption was identified in central areas, whereas proximal areas

yielded only minor changes. Thin-wall phenotypes displayed pronounced vertical bone resorption, with a median bone loss of 7.5 mm, as compared with thick-wall phenotypes, which decreased by only 1.1 mm.

Takahashi Y, Marukawa E, Omura K (2013)[29] conducted study on β-TCP/collagen composites in extraction socket preservation in an experimental study in dogs. Following the extraction of the maxillary second and third premolars of 13 beagle dogs, a bone defect with buccal dehiscence (5 × 3 × 7 mm) was prepared. The defects were filled with either TCP/collagen or left intact (control) and evaluated at 4 and 8 weeks after surgery. Newly formed bone and residual TCP in the bone defect site of the specimens was also measured and evaluated with the help of micro-computed tomography. Results exhibited that the TCP/Col composites could sufficiently maintain bone width and height for the preservation of the extraction socket with buccal dehiscence while preventing epithelial in-growth.

Suttapreyasri S , Leepong N (2013)[30] investigated the influence of platelet-rich fibrin (PRF) on early wound healing and preservation of the alveolar ridge shape following tooth extraction. In this clinical trial, 20 symmetrical, premolar extraction sockets using split-mouth design were randomly selected with PRF or blood clot. The evaluations of wound healing, alveolar ridge contour changes, and crestal bone resorption were performed in dental casts and periapical radiographs (T0, initial; T1, 1 week; T2, 2 weeks; T4, 4 weeks; T6, 6 weeks; T8, 8 weeks). Results showed that platelet-rich fibrin clinically showed early healing of soft tissue covering socket orifices in the first 4 weeks. At the first week, the horizontal resorption on buccal aspect of PRF (1.07 ± 0.31 mm) was significantly less than that of

the control (1.81 ± 0.88 mm). This preliminary result demonstrated neither better alveolar ridge preservation nor enhanced bone formation of PRF in the extraction socket. The use of PRF revealed limited effectiveness by accelerated soft-tissue healing on the first 4 weeks.

Hauser F, Gaydarov N, Badoud I, Vazquez L, Bernard JP, Ammann P (2013)[31] investigated whether the use of platelet-rich fibrin membranes (PRF) for socket filling could improve microarchitecture and intrinsic bone tissue quality of the alveolar bone after premolar extraction and to assess the influence of the surgical procedure before implant placement. Twenty-three patients requiring premolar extraction followed by implant placement were randomized to three groups: (1) simple extraction and socket filling with PRF, (2) extraction with mucosal flap and socket filling with PRF, and (3) controls with simple extraction without socket filling. Implant placement was performed at week 8, and a bone biopsy was obtained for histomorphometric analysis.

Analysis by microcomputed tomography showed better bone healing with improvement of the microarchitecture ($P < 0.05$) in group 1. This treatment had also a significant effect ($P < 0.05$) on intrinsic bone tissue quality and preservation of the alveolar width. An invasive surgical procedure with a mucosal flap appeared to completely neutralize the advantages of the PRF. Results support the use of a minimally traumatic procedure for tooth extraction and socket filling with PRF to achieve preservation of hard tissue.

S. Girish Rao, Preethi Bhat, K. S. Nagesh (2013)[32] conducted a study to evaluated the effects of autologous platelet rich fibrin gel (PRF gel) on bone regeneration

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S. Girish Rao, Preethi Bhat, K. S. Nagesh (2013)[32] conducted a study to evaluated the effects of autologous platelet rich fibrin gel (PRF gel) on bone regeneration

following extraction. Study sample consisting of a total of 22 patients requiring bilateral transalveolar third molar extractions. One side was randomly chosen as case and the other side was the control. The PRF gel was placed in the extraction site and primary closure was obtained. The Regeneration of bone was measured and compared using serial radiographs (RVG) at immediate post op, one, three and six months. RVGs were assessed for amount of radiologic bone filling by the method described by Matteo Chiapasco et al. Higher mean pixels was recorded in cases compared to controls at all the time intervals.

Hatakeyama I, Marukawa E, Takahashi Y, Omura K (2014)[33] conducted a study to evaluated the effects of platelet-poor plasma (PPP), platelet-rich plasma (PRP), and platelet-rich fibrin (PRF) on healing in a ridge-augmentation model of the canine socket with dehiscence of the buccal wall. The third mandibular premolars of 12 beagle dogs were extracted and a 3 mm buccal dehiscence from the alveolar crest to the buccal wall of the extraction socket was created. These sockets were then divided into four groups on the basis of the material used to fill the sockets: PPP, PRP, PRF, and control (no graft material) groups. A total of five microcomputed tomography images of specimens were selected for measurement. The median area of new bone at 4 and 8 weeks and median horizontal bone width at 8 weeks were the highest in the PPP group. The growth factors released from platelets in PRP indicated higher concentrations than that in PRF. Under more severe conditions for bone formation, as in this experiment, the growth factors released from platelets had a negative effect on bone formation. This study showed that PPP is an effective material for the preservation of sockets with buccal dehiscence.

Naik B, Karunakar P, Jayadev M, Marshal VR (2014)[34] conducted a study aimed at reviewing and discussion the strategies available for use of PRF as healing aid in dentistry. It was concluded that by showing good promising results using PRF, it has proved to have a good prospect for its use as healing aid in various aspects of dentistry. PRF can be considered as an immune concentrate with specific composition and a tree dimensional sructure.It contains growth factor like platelet derived growth factor, Transforming growth factor, Insulin like growth factor.It is biocompatible, easy to prepare and cost effective and provide a slowly release of growth factor for a long time.

Raja VS, Naidu EM (2014)[35] conducted a study which reviewed a description of the evolution of the novel platelet concentrate, referred to as PRF. It covered PRP preparation and properties and concluded that popularity of this material should increase considering its many advantages, though more histologic evaluations are required to understand other benefits. It is cost effective and easy to prepare and it releases growth factors slowly and helps in bone formation.

Saluja H, Dehane V, Mahindra U (2014)[36] conducted a study which describes the evolution of this second generation platelet concentrate and its multiple uses in various surgical procedures. This article concluded that PRF can be used for all types of superficial cutaneous and mucous healing. Fibrinogen is the final substrate of all coagulation reaction. Being a soluble protein, it is transformed into insoluble fibrin by thrombin, while the polymerized fibrin gel constitute the first cicatrical matrix of the injured site.

Panda S, Jayakumar ND, Sankari M, Varghese SS, Siva Kumar D (2014)[37] conducted a study case report aimed to investigate the clinical and radiological (bone fill) effectiveness of autologous PRF along with the use of xenogenic bone mineral. A decrease in PPD, gain in CAL and significant bone fill was observed at the end of 6 months. PRF has many advantages over PRP. It eliminates the redundant process of adding anticoagulant as well as the need to neutralize it. The addition of bovine-derived thrombin to promote conversion of fibrinogen to fibrin in PRP is also eliminated. The elimination of these steps considerably reduces biochemical handling of blood as well as risks associated with the use of bovine-derived thrombin.

Naik B, Karunakar P, Jayadev M, Rahul Marshal V (2014)[38] conducted a study which aim was to review and discuss the strategies available for use of platelet rich fibrin as healing aid in dentistry. It was concluded that by showing good promising results with use of the PRF, it proved to have a good prospect for its use as healing aid in various aspects of the dentistry.

Shetty SK, Pradeep AR, Deshmukh VL, Acharya A (2014)[39] conducted a study and aim of the study was to study the clinical effectiveness of autologous PRP in treating intrabonny defects in humans in promoting wound healing, reduction in PPD, gain in CAL and bone fill in intrabony defects. Treatment by PRP gel alone caused significant soft tissue clinical improvement as well as hard tissue defect fill. Due to its peculiar properties, the natural fibrin biomaterial PRF has a great potential for surgical wound healing and shown to be an effective regenerative material in the management of grade 2 furcation involvement.

Vinaya Kumar R, Shubhashini N (2014)[40] conducted a study served as an introduction to the PRF "concept" and its potential clinical applications with emphasis on periodontal regeneration. This second generation platelet concentrate called Platelet rich fibrin (PRF), has been widely used to accelerate soft and hard tissue healing. Its advantages over the better known PRP include ease of preparation/application, minimal expense, and lack of biochemical modification (no bovine thrombin or anticoagulant is required.

Agarwal M, Agarwal V (2014)[41] conducted a study to describe PRF and its applications in dentistry. It was concluded that the use of PRF as an adjunct in wound healing and periodontal regeneration has shown promising results. Also, in addition to clinical trials, histopathological studies are also required to learn about the nature of the newly formed tissuein the defect and to understand the biology, efficacy and mode of action of PRF more effectively.

.Piemontese M, Aspriello SD, Corrado R, Ferrantte L, Procaccini M (2015)[42] concluded that a combination of Platelet Rich Plasma (PRP) and DFDBA led to a significantly greater clinical improvement in intrabony defects compared to DFDBA with saline. According to their study there was no statistically significant differences a were observed in the hard tissue response between the two treatment groups, which confirmed that PRP had no effect on hard tissue fill or gain in new hard tissue formation.

Ilgenli T, Dundar N, Ilhan Kal B (2015)[43] conducted a study was to compare the clinical and radiographic outcomes of DFDBA/PRP combination with PRP alone for the treatment of infrabony defects 18 months post-surgery and to examine the

influence of radiographic defect angle on the clinical and radiographic outcomes. The results indicated that DFDBA/PRP combination is more effective than PRP alone for the treatment of infrabony defects, and the amount of CAL gain, PPD reduction, and bone fill increases when the infrabony defect is narrow and deep before DFDBA/PRP combination treatment.

Bhedasgaonkar SY, Kapadia J, Patil NA (2015)[44] conducted a study aimed at comparing the clinical and radiographical outcome obtained by combination of PRF and allograft including case reports of 2 patients with infrabony defects treated with PRF combined with allograft. It was concluded that PRF is efficacious clinically and radiographically in periodontal infrabony defect. PRF is a concentrated suspension of growth factor founds in platelets which moderate the wound healing and promote tissue regenaration.They evaluates the clinical efficacy PRF with DFDBA allogaraft in the treatment of infrabony defects in patients with chronic periodontitis and showed a significant improvement in clinical and radiological parameters.

Materials & Methods

A 4- month experimental prospective clinical study was carried out in the Department of Periodontics, Babu Banarasi Das College of Dental Sciences (BBDCODS), Lucknow to evaluate the effectiveness and clinical outcome of combination of Platelet Rich Fibrin (PRF), with β- Tricalcium phosphate as a graft material and PRF alone for alveolar ridge preservation in extraction socket. For radiographic analysis CBCT was done at a private diagnostic center.

A total of 20 patients irrespective of gender, suffering from chronic periodontitis with grade-III mobility or having grossly decayed non restorable tooth were selected from the O.P.D of the department. An appropriate clearance from the Institutional Ethics Committee was taken for the study. Patients were clearly explained the study protocol and procedure. A duly signed written consent was taken from them. A strict inclusion and exclusion criteria was followed for the recruitment of the subjects.

PATIENT SELECTION:

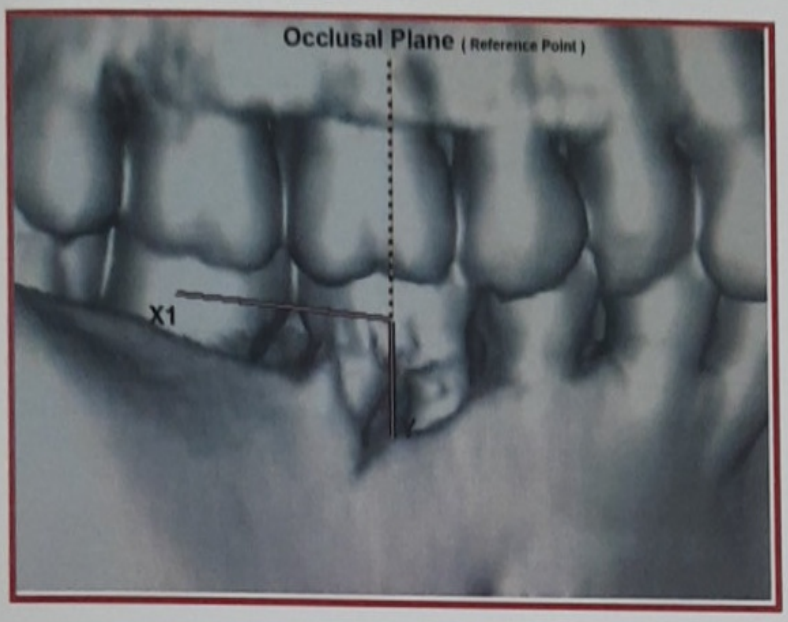
At the time of screening medical and dental history was obtained. The selection criteria were based upon the following inclusion and exclusion criteria:

❖ **INCLUSION CRITERIA:**

1. Adults of both gender of age range between 28-55 years.
2. Patients with good general health without any history of significant systemic diseases.
3. Teeth with grade III mobility of teeth with hopeless prognosis.
4. Grossly decayed tooth which cannot be restored and indicated for extraction.

RADIOGRAPHIC RIDGE MAPPING PARAMETERS

BUCCAL & LINGUAL VERTICAL BONE HEIGHT



HORIZONTAL BONE WIDTH

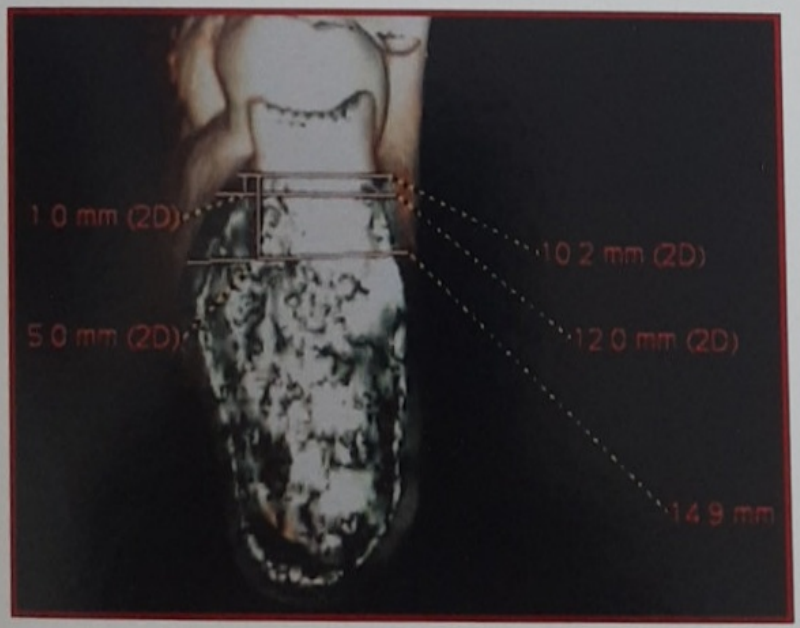


PLATE NO. I

❖ **EXCLUSION CRITERIA:**

1. Smokers, tobacco and/ or pan masala chewers, alcoholics, drug addicts.
2. Pregnant and lactating women.
3. Person having systemic diseases or conditions that influence the progression and/or clinical characteristics of periodontal disease.
4. Teeth with no periapical pathology.
5. Subjects allergic or sensitive to any medication.
6. Subject on asprin or any other anti-coagulant therapy which might interfere with the coagulation while preparing PRF.

STUDY DESIGN:

The clinical study was conducted in the Department of Periodontics, Babu Banarasi Das College of Dental Sciences, Lucknow. It was a randomised, experimental and a longitudinal study, where intragroup and intergroup comparison was made for the two groups at baseline and at four months postoperatively. Radiographic analysis by CBCT was done at a private diagnostic centre. The enrolment of volunteers, their clinical examinations and blood collection was done at BBDCODS, Lucknow.

GROUP DESCRIPTION:

The volunteers were randomly divided into 2 groups after the diagnosis and out of 20 extraction sites, 10 sites were selected for PRF in combination with Beta Tri-Calcium and 10 sites for PRF alone.

ARMAMENTARIUM FOR DIAGNOSIS , SCALING & ROOT PLANING

PLATE NO. II

Group A: PRF alone

Group B: Combination of PRF and β-TCP.

CLINICAL PARAMETERS:

Evaluation of alveolar ridge dimension was done with the help of CBCT. The following radiological parameters were recorded to the nearest $1/10^{th}$ of mm with the help of a CBCT by a single investigator for each site before surgery (baseline) and postoperatively at 4 months.

RADIOGRAPHIC MEASUREMENTS:

ALVEOLAR RIDGE DIMENSIONS ASSESSED BY CBCT

CBCT was performed with subjects in a supine position. Cotton roll was placed in the mouth between upper and lower teeth to prevent overlapping of the occlussal plane. To minimize exposure dose and time the CBCT images (EYE CAT) were obtained from 5-mm axial sections, which was reconstructed to 0.5 mm axial sections by the inbuilt software. Sagittal or coronal cross sections were also processed by the software program provided by the manufacturer. All the measurements were performed using the software's built-in measurement too (Advantage Workstation, ADW-4.6) by single investigator. The largest distance between the buccal and lingual bone walls was recorded to the nearest 0.1 mm.

ARMAMENTARIUM FOR PRF PREPARATION

CENTRIFUGE

PLATE NO. III

RIDGE-MAPPING PARAMETERS

Ridge dimensional analyses were performed on the CBCT to measure:

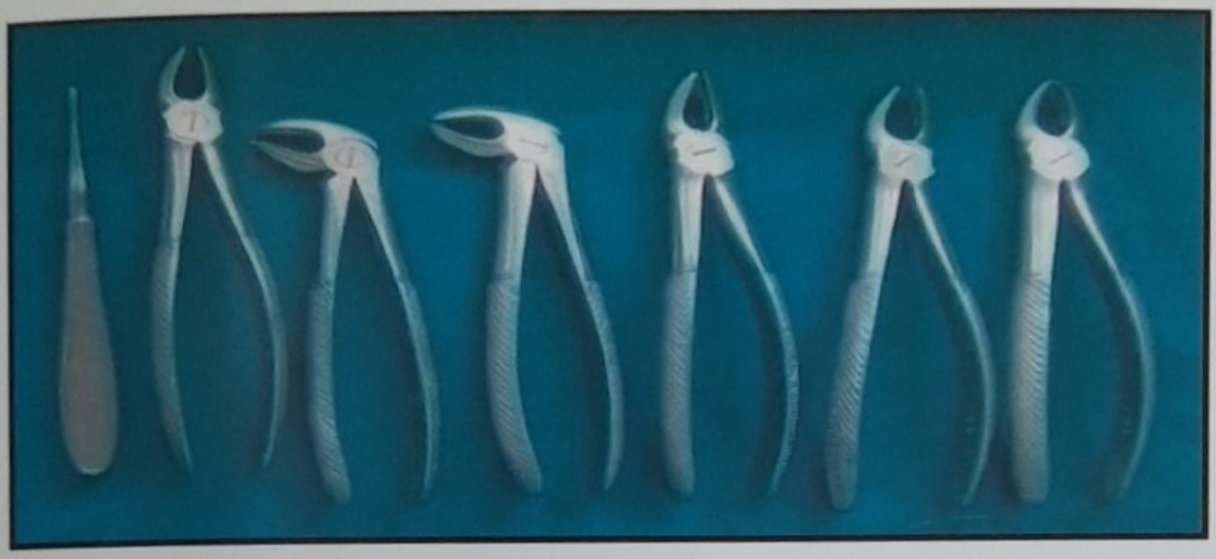
1. **Buccal and Lingual Cortical Bone Height**: With the help of inbuilt software, virtual three dimensional (3D) image of the jaw bone was constructed. Vertical height of buccal and lingual/palatal socket wall in coronal view was measured over this 3D image. Occlusal plane was taken as a reference points. A horizontal line (X_1) was drawn from one occlusal plane to another of the adjacent teeth. From the most apical position of cortical bone a line (Y) was drawn coronally towards the horizontal reference line (X_1). To make this measurement repeatable in the second CBCT a second parallel horizontal line (X_2) was drawn from the occlusal surface either of reference points till the meeting point of X_1 and Y. Any reduction in this 'Y' measurement from pre treatment to post treatment indicates gain in vertical bone height and referred to bone augmentation. For this study the measurements were done the measurement was done from the occlusal line. Non significant changes indicated successful preservation of alveolar ridge at its previous position prior extraction.

2. **Bucco-lingual/palatal width of alveolar bone** : It was measured at mid crest region in a axial view at crest of the ridge 0, 1 and 5 mm apical to the crest recorded to the nearest millimetre. Any reduction in this measurement from pre treatment to post treatment indicated actual loss of alveolar ridge width.

PRESURGICAL PROCEDURES:

Each individual was subjected to

ARMAMENTARIUM FOR TOOTH EXTRACTION



PERIOTOME

PLATE NO. IV

1. A detailed case history record.

2. A duly informed consent of each patient was taken for the purpose of the study.

3. Intra-oral periapical (IOPA) X-rays was done at BBDCODS for initial screening for patient selection

4. Dental CBCT was done at private diagnotic centre, Lucknow at base line and 4 months after treatment.

5. Routine lab investigations –

- Complete blood count
- Blood sugar (random)
- Test for HIV I & HIV II

MATERIAL:

Armamentarium for Diagnosis & Scaling and Root Planing:

Armamentarium for PRF Preparation:

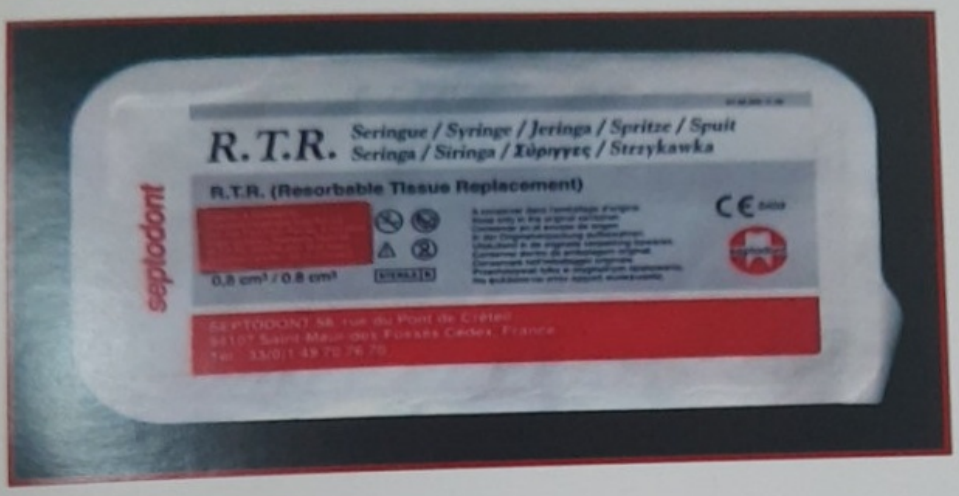
- Surgical spirit
- Cotton
- Tourniquet
- Disposable plastic syringe: 20 ml
- Sterilized glass test tubes
- Centrifuge machine (Forco Scientific Udyog Pvt. Ltd, India)

Armamentarium for Atraumatic Tooth Extraction:

ARMAMENTARIUM FOR SURGICAL PROCEDURE

PLATE NO. V

β- TRICALCIUM PHOSPHATE BONE GRAFT



BARRIER MEMBRANE

PLATE NO. VI

- Periotome (Hu-Friedy, USA)
- Extraction forceps (API, Germany)
- Elevators (API, Germany)

Armamentarium for Surgical Procedur:

- Local anaesthetic agent 2% Lignocaine HCl containing (1:80,000) epinephrine
- β- TCP bone graft (Septodont)
- Barrier membrane (BioMesh-S [DMB8000] SAMYANG, Korea)
- Bone Graft Condenser
- Syringe 3ml and 5 ml
- Normal saline
- Sterilized cotton, Gauze, Cheek retractor
- Povidone –Iodine solution 5% w/v
- Bard-Parker handles (No.3)
- B.P. Blade Nos. 11, 12 and 15.
- Periosteal elevators
- Adams tissue holding forcep
- A set of surgical curettes
- Castroviejo scissors
- Pair of straight and curved conventional Gold man fox scissors
- Castroviejo needle holder/Needle Holder
- Sutures (4-0) (non-resorbable braided silk and needle)

PRE OPERATIVE PHOTOGRAPH

IOPA X-RAY

PLATE NO VII

- Suture cutting scissors
- Coe-pack for dressing.

CLINICAL TREATMENT PROCEDURE:

Patients underwent the following pre-surgical procedures:

PHASE I THERAPY:

All the selected subjects underwent phase-I therapy which included oral hygiene instructions, full mouth supra and sub gingival scaling and root planing and occlusal correction if trauma from occlusion existed. Scaling and root planing were performed using hand and ultrasonic instruments. Adjunctive chemical plaque control, in the form of chlorhexidine mouth rinse 0.2% twice daily, was advised and patients were re-evaluated for plaque control after 1 week.

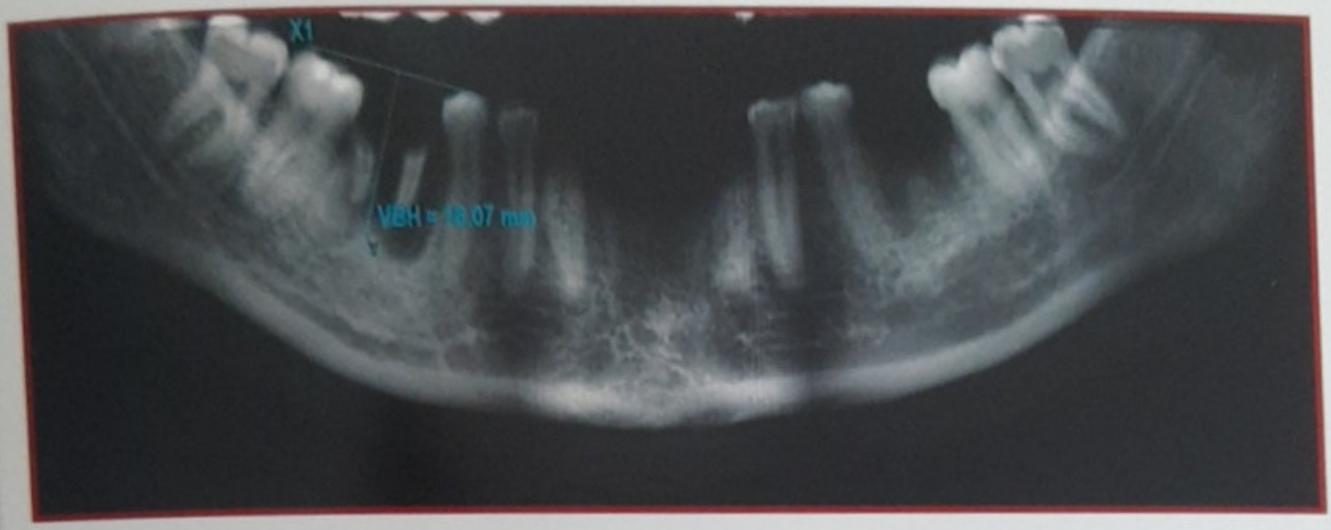
The patients were taken to private diagnostic center for CBCT to get preoperative radiological parameters.

SURGICAL PROCEDURE:

Preparation of PRF

The 10 ml blood was drawn from the median cubital vein that lies within the cubital fossa. 10 systemically healthy volunteers of age group 28 yrs to 55 yrs at Babu Banarasi Das College of Dental Sciences, Lucknow, into a sterile glass tubes without anticoagulant and centrifuged immediately. The centrifugation process was carried

PRE OPERATIVE CBCT OF VERTICAL BONE HEIGHT



PRE OPERATIVE CBCT HORIZONTAL WIDTH AT 0 MM

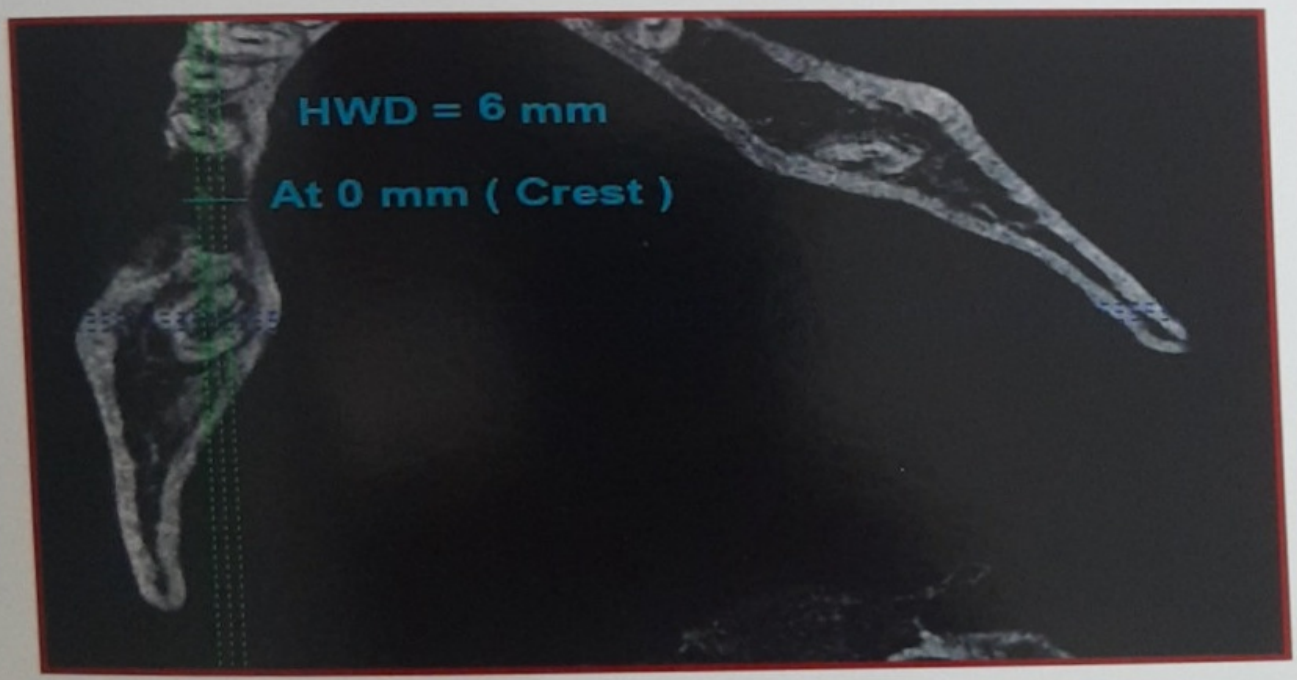
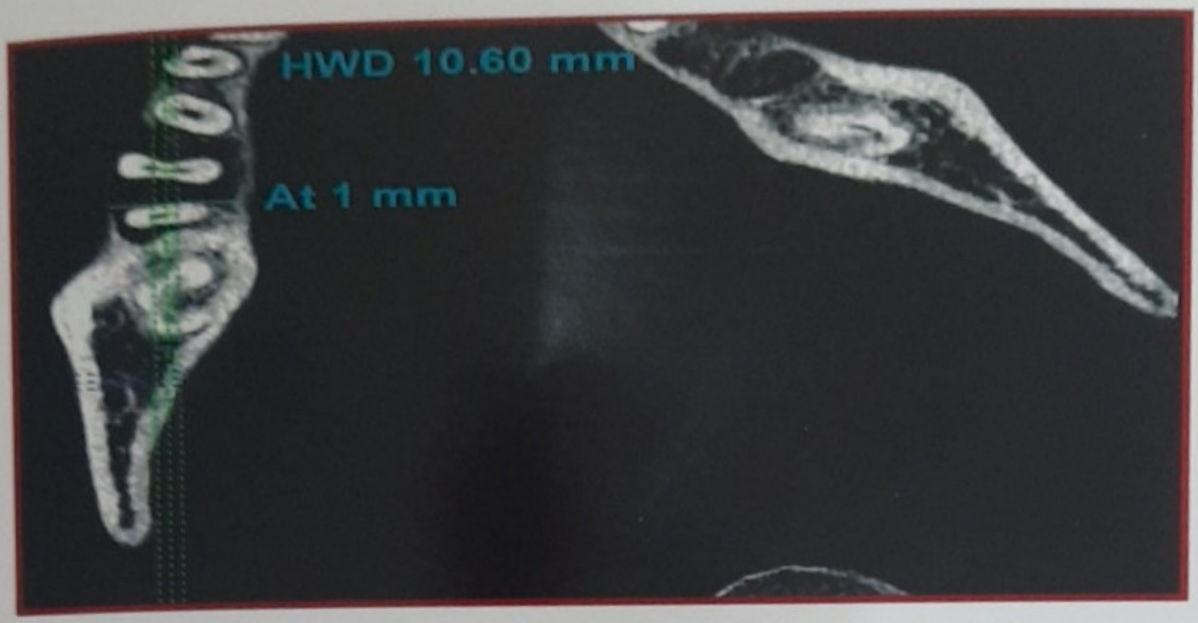


PLATE NO. VIII

PRE OPERATIVE CBCT
HORIZONTAL WIDTH AT 1 MM



PRE OPERATIVE CBCT
HORIZONTAL WIDTH AT 5 MM

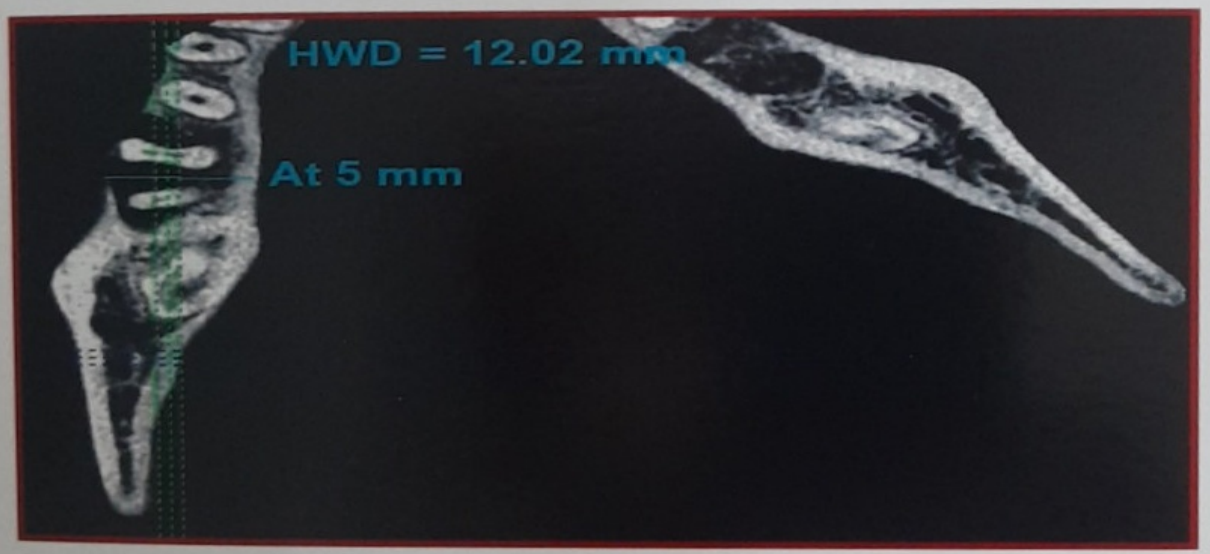


PLATE NO. IX

out as per the protocol described by Dohan et al with certain modifications. In brief, the tubes were immediately centrifuged in Laboratory centrifuge at 3,000 rpm for 15 minutes at room temperature. The tubes were then kept in rest at room temperature for few minutes, so that the platelets could trigger the coagulation cascade in the absence of anticoagulant. In first instance, fibrinogen concentrates in the upper part of the tube, followed by the circulating thrombin which transforms fibrinogen into a fibrin network. Finally, a fibrin clot containing the platelets was obtained in the middle of the tube, between top plasma and bottom red blood cell layer. The PRF was isolated using tweezers and then cut by scissors and placed in sterile normal saline till it was packed in socket.

EXTRACTION OF TOOTH:

Routine preparation of the patient with 5% w/v Povidone Iodine solution was carried out. Following the administration of local anesthesia, crevicular incisions were made around the tooth to be extracted followed by two vertical incision on either side up to mucogingival junction and full thickness mucoperiosteal flap were reflected buccally to obtain complete access to the crestel bone and to facilitate coronal advancement of the flap. Periotome was used to break periodontal attachment and tooth was extracted atraumatically. Careful debridement of granulation tissue if any, from extraction socket was done with surgical curette and throughly irrigated with normal saline

PRF and β- TCP GRAFT PLACEMENT:

PERIPHERAL BLOOD COLLECTION

PRF AFTER CENTRIFUGATION

PLATE NO. X

SURGICAL PROCEDURE

PRF with β-TCP GRAFT PLACEMENT

MEMBRANE PLACEMENT

SUTURE APPLICATION

COE-PACK APPLICATION

PLATE NO XI

PRF and β- TCP granule (Septodont) was condensed with a condenser inside the socket. Resorbable barrier membrane (BioMesh-S [DMB8000] SAMYANG, Korea) was placed over the grafted material. The flap was coronally advanced for close approximation and then 4-0 non-resorbable silk sutures were used to approximate the lingual and buccal flaps. The surgical area was protected and covered with periodontal dressing (Coe Pack).

Patients were kept on antimicrobial therapy amoxicillin 500mg, 1 cap TDS for 5 days along with anti-inflammatory drug. Chlorhexidine 0.2% twice daily for 2 weeks was prescribed as mouthwash. Other post surgical instruction were given in writing and patient were recalled after 1 week for evaluation. Sutures and coe-pack were removed after 2 week. They were kept on maintenance phase and scaling and root planing was carried out as and when required.

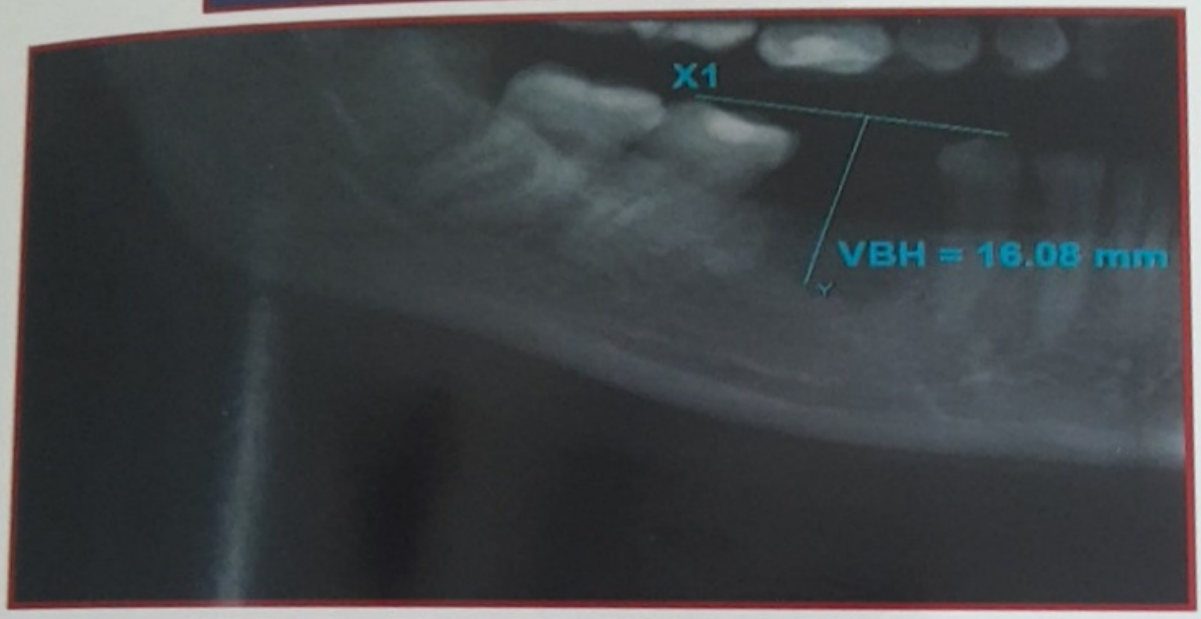
EXTRACTION OF TOOTH AND PRF PLACEMENT:

Prior to extraction PRF preparation was done.

PRF placement inside the socket

Similar surgical procedure was done for PRF group as was performed for combination of PRF and β- TCP group. PRF was placed in the extraction socket for PRF group. Since PRF is an autologous graft and resembles natural clot therefore acted both as graft and a membrane. So no other membrane was used as barrier.

4 MONTHS POST OPERATIVE CBCT OF VERTICAL BONE HEIGHT



4 MONTHS POST OPERATIVE CBCT HORIZONTAL WIDTH AT 0 MM

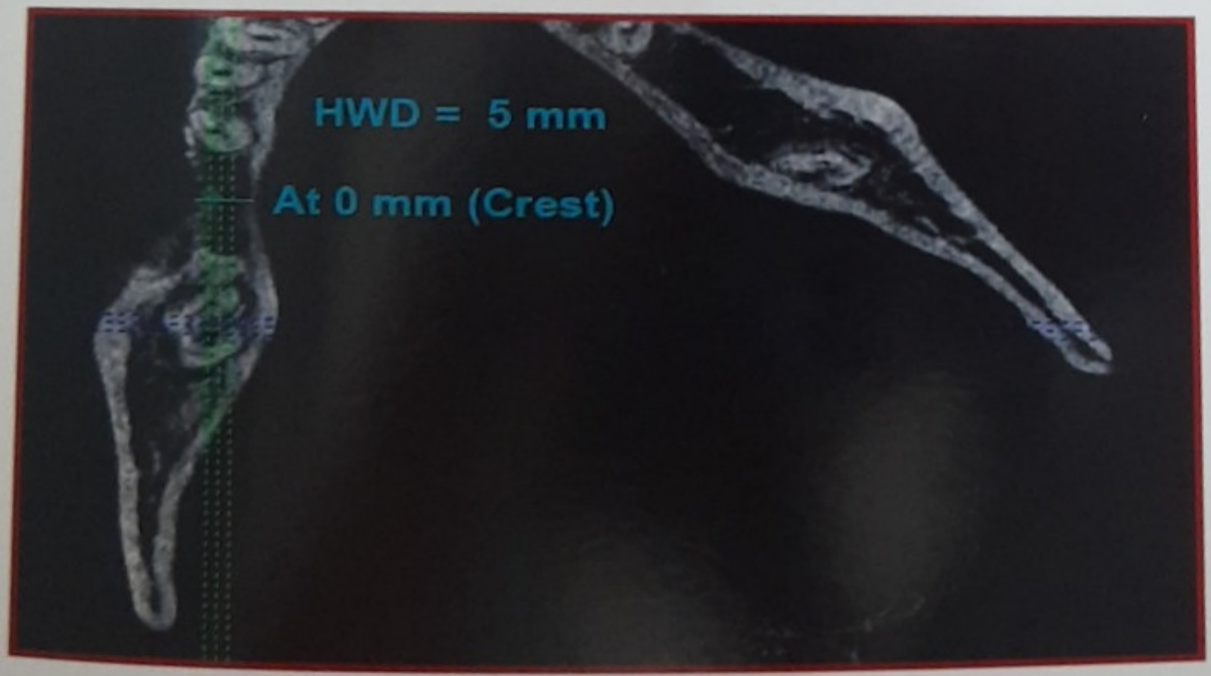
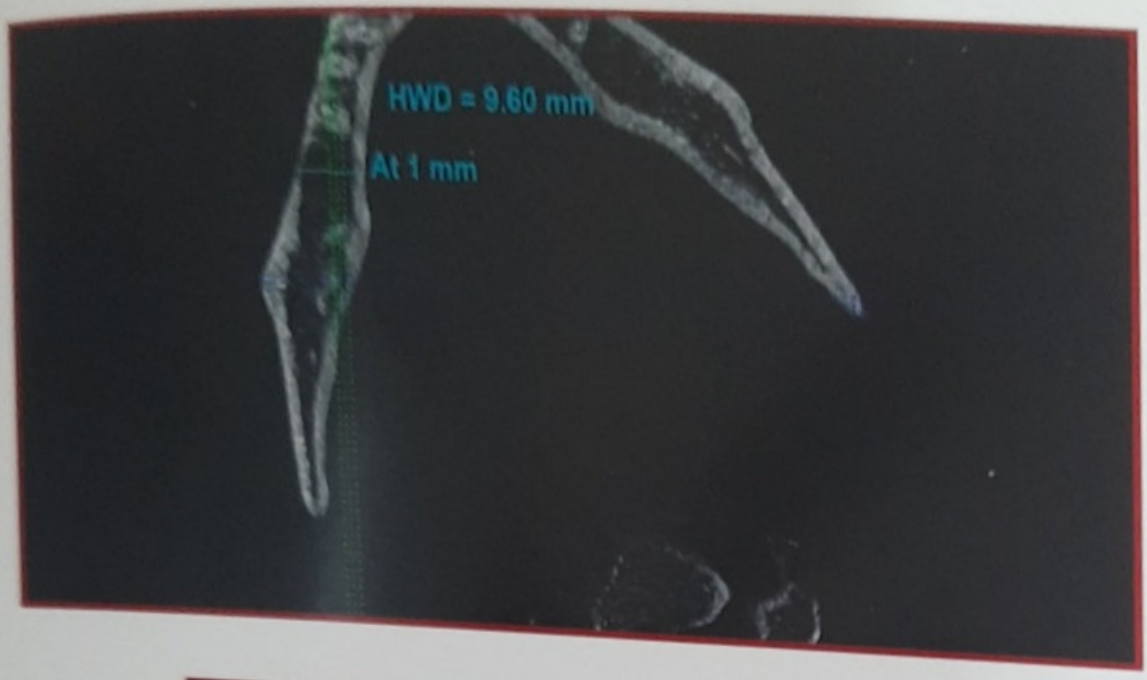


PLATE NO. XII

4 MONTHS POST OPERATIVE CBCT HORIZONTAL WIDTH AT 1 MM



4 MONTHS POST OPERATIVE CBCT HORIZONTAL WIDTH AT 5 MM

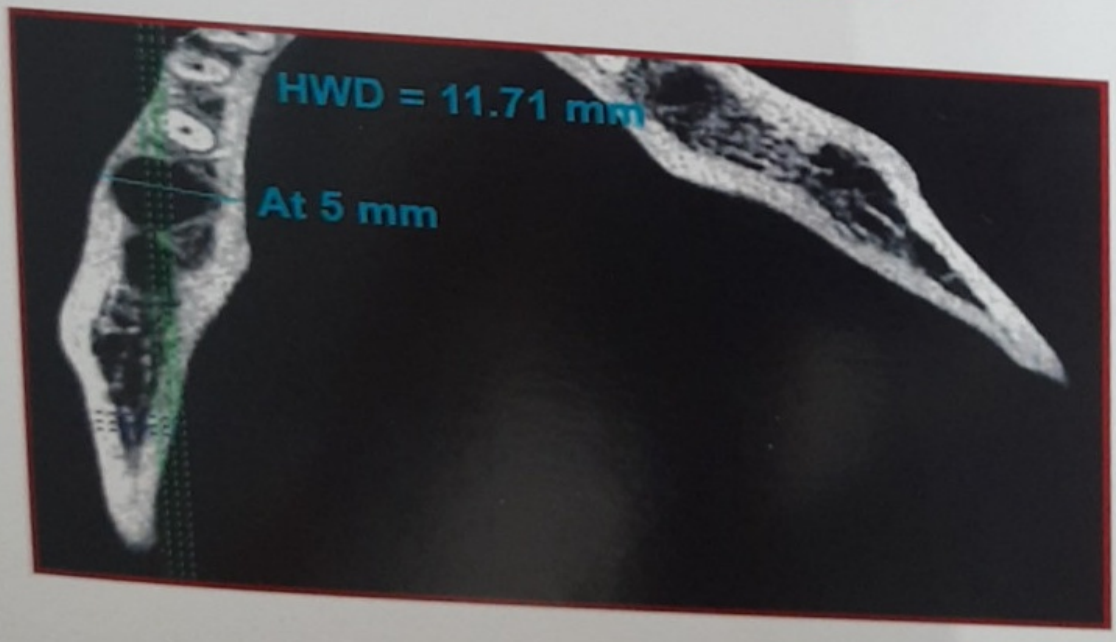


PLATE NO. XIII

SURGICAL PROCEDURE- PRF GROUP

EXTRACTION

PRF PLACEMENT

SUTURE APPLICATION

PLATE NO. XIV

All the medications, instructions and post surgical procedures remained the same.

For all the subjects another CBCT of jaw was done 4 months post treatment.

Statistical analysis

Data were summarized as Mean ± SD. Groups were compared by paired t test. Groups were also compared by independent Student's t test. A two-tailed (α=2) p value less than 0.05 ($p<0.05$) was considered statistically significant. All analyses were performed on SPSS software.

Results & Observations

The present clinico-radiograph study evaluates and compares the platelet rich fibrin alone (Group A) and PRF with β-Tricalcium(Group B) phosphate bone graft for alveolar ridge preservation in extraction socket. Total 20 patients, age between 28-55 yrs, either sex, suffering from chronic periodontitis with grade-III mobility or having grossly decayed non restorable tooth were selected and randomized equally into two groups and treated either with platelet rich fibrin alone (PRF) or platelet rich fibrin & β-Tricalcium phosphate (PRF & β-TCP) (Table 1 and Fig. 1). The outcome measures of the study were vertical bone height (buccal and lingual) and axial bone width (0 mm, 1 mm and 5 mm) assessed at pre treatment (pre) and 4 month post treatment (post) measured in millimeters (mm). The objective of the study was to compare the socket preservation between the two groups (PRF and PRF & β-TCP).

Table 1: Group allocation and distribution of patients in each group

Treatment/graft material	Groups	Group name	No of patients (n=20) (%)
Platelet rich fibrin	Group A	PRF	10 (50.0)
Platelet rich fibrin & β-TCP	Group B	PRF & β-TCP	10 (50.0)

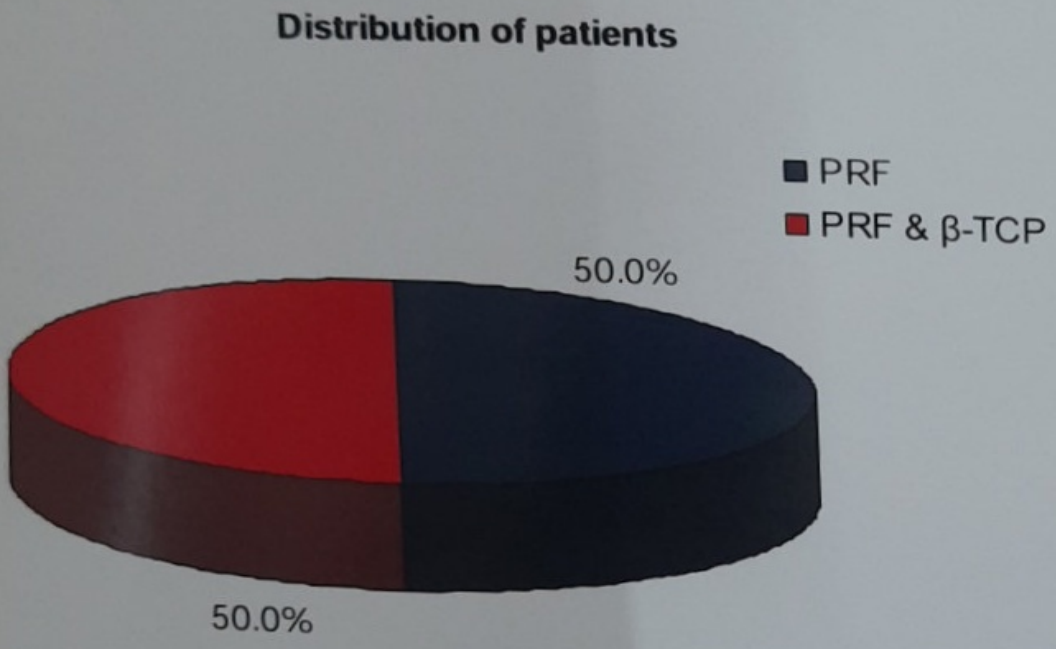


Fig.1. Distribution of patients of two groups.

Outcome measures

A. Vertical bone height

1. Buccal

The pre and post vertical bone height of two groups (PRF and PRF & β-TCP) at buccal side is summarized in Table 2. In PRF group, the pretreatment mean (± SD) vertical bone height was 14.14 ± 3.89 mm while at post it was 13.66 ± 3.83 mm. Comparing the pre and post mean vertical bone height, paired t test showed significant change/gain (0.48 ± 0.37 mm) in vertical bone height at post as compared to pre (14.14 ± 3.89 vs. 13.66 ± 3.83, t=4.10, p=0.003) (Table 2 and Fig. 2).

Similarly, in PRF & β-TCP group, the pretreatment mean (± SD) vertical bone height was 14.35 ± 3.19 mm while at post it was 13.45 ± 3.11 mm. Comparing the pre and post mean vertical bone height, paired t test showed significant change/gain (0.90 ± 0.46 mm) gain in vertical bone height at post as compared to pre (14.35 ± 3.19 vs. 13.45 ± 3.11, t=6.13, p<0.001) (Table 2 and Fig. 3).

Further, comparing the socket preservation (i.e. mean change from pre to post) in vertical bone height of two groups at buccal side, Student's t test showed significantly different and higher (46.7%) socket preservation in vertical bone height in PRF & β-TCP group as compared to PRF group (0.48 ± 0.37 vs. 0.90 ± 0.46, t=2.24, p=0.038) (Table 2 and Fig. 4).

Table 2: Pre and post vertical bone height (Mean ± SD) of two groups at buccal side

Groups	Pre (n=10)	Post (n=10)	Change (Pre-Post)	t value	p value
PRF	14.14 ± 3.89	13.66 ± 3.83	0.48 ± 0.37	4.10	0.003
PRF & β-TCP	14.35 ± 3.19	13.45 ± 3.11	0.90 ± 0.46	6.13	<0.001

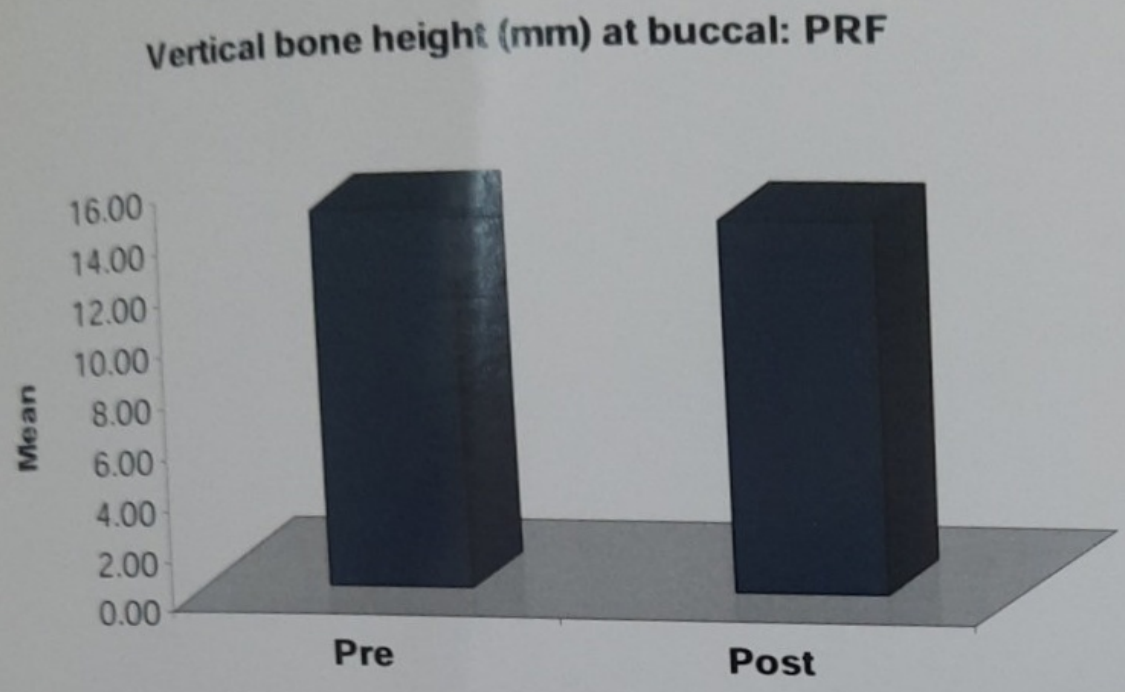


Fig. 2. Pre and post vertical bone height of PRF group at buccal side.

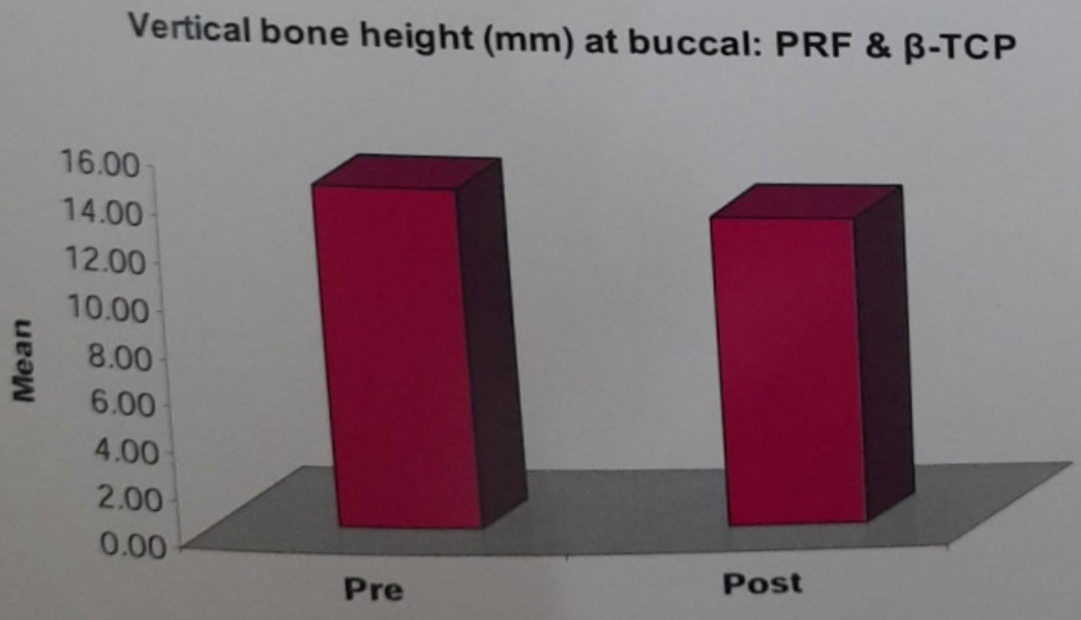


Fig. 3. Pre and post vertical bone height of PRF & β-TCP group at buccal side.

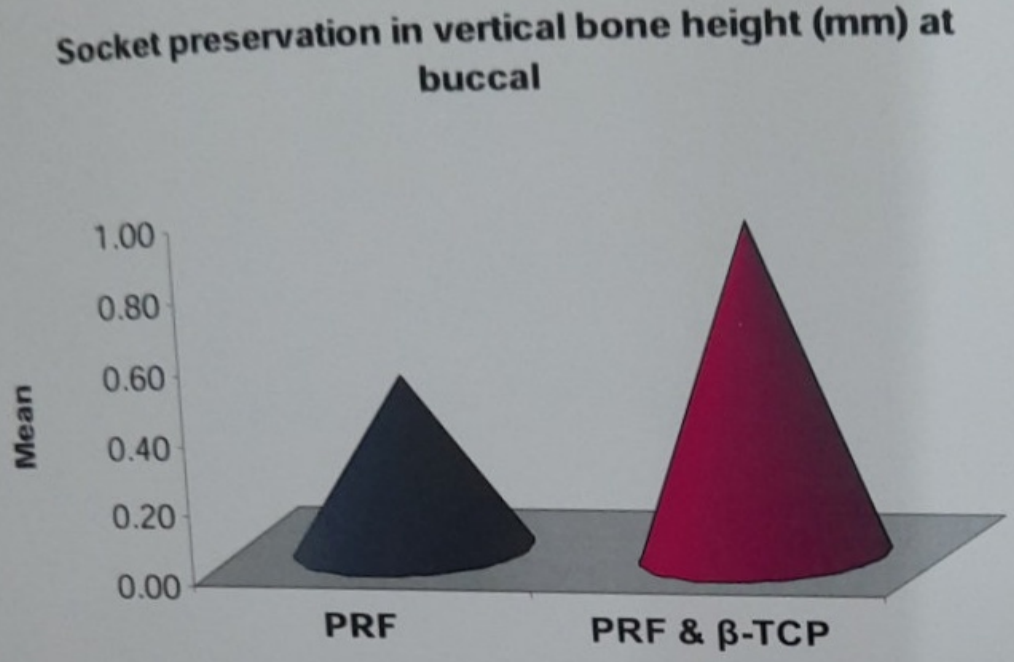


Fig. 4. Socket preservation in vertical bone height of two groups at buccal side.

II. Lingual

The pre and post vertical bone height of two groups (PRF and PRF & β-TCP) at lingual side is summarized in Table 3. In PRF group, the pretreatment mean (± SD) vertical bone height was 12.34 ± 2.80 mm while at post it was 11.70 ± 2.75 mm. Comparing the pre and post mean vertical bone height, paired t test showed significant change/decrease (0.64 ± 0.35 mm) or gain in vertical bone height at post as compared to pre (12.34 ± 2.80 vs. 11.70 ± 2.75, t=5.83, p<0.001) (Table 3 and Fig. 5).

Similarly, in PRF & β-TCP group, the pretreatment mean (± SD) vertical bone height was 12.29 ± 2.98 mm while at post it was 11.31 ± 2.93 mm. Comparing the pre and post mean vertical bone height, paired t test showed significant change/decrease (0.98 ± 0.29 mm) or gain in vertical bone height at post as compared to pre (12.29 ± 2.98 vs. 11.31 ± 2.93, t=10.69, p<0.001) (Table 3 and Fig. 6).

Further, comparing the mean socket preservation (i.e. mean change from pre to post) in vertical bone height of two groups at lingual side, Student's t test showed significantly different and higher (34.7%) socket preservation in vertical bone height in PRF & β-TCP group as compared to PRF group (0.64 ± 0.35 vs. 0.98 ± 0.29, t=2.38, p=0.029) (Table 3 and Fig. 7).

Table 3: Pre and post vertical bone height (Mean ± SD) of two groups at lingual side

Groups	Pre (n=10)	Post (n=10)	Change (Pre-Post)	t value	p value
PRF	12.34 ± 2.80	11.70 ± 2.75	0.64 ± 0.35	5.83	<0.001
PRF & β-TCP	12.29 ± 2.98	11.31 ± 2.93	0.98 ± 0.29	10.69	<0.001

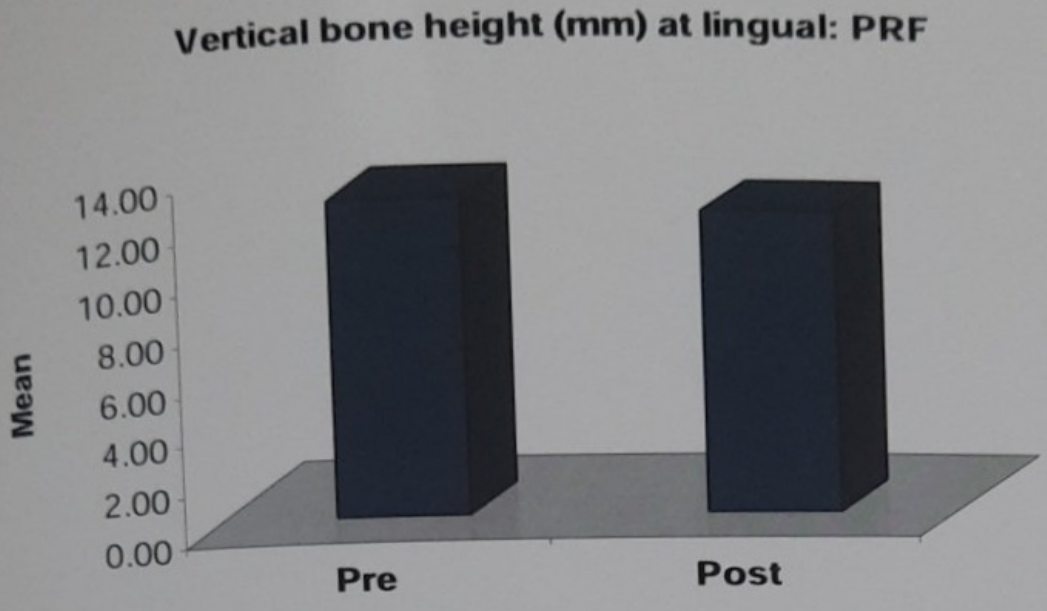


Fig. 5. Pre and post vertical bone height of PRF group at lingual side.

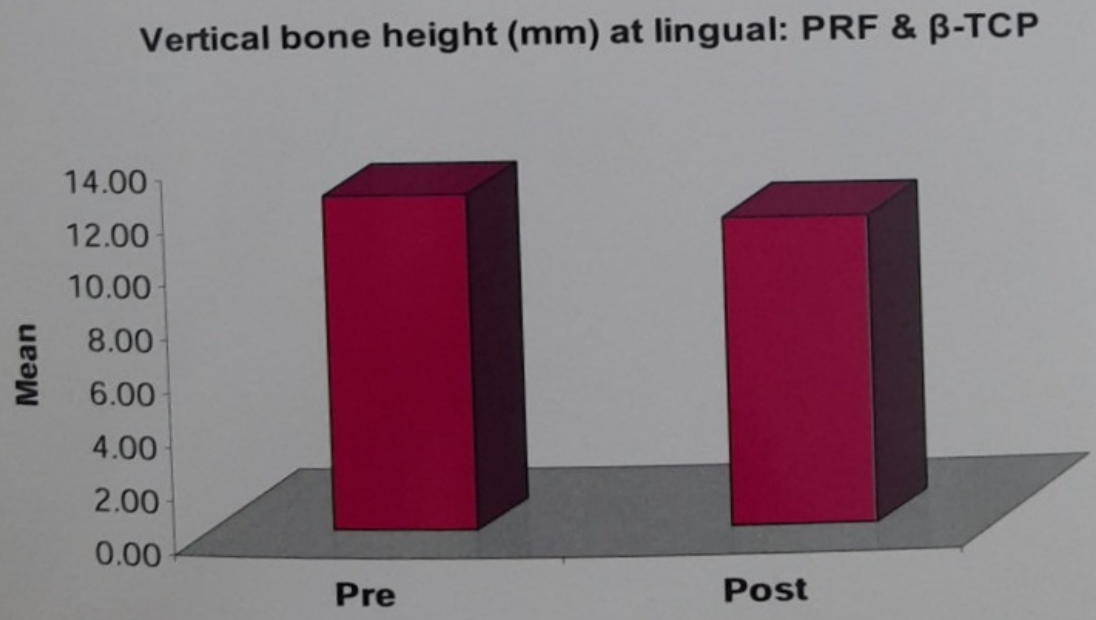


Fig. 6. Pre and post vertical bone height of PRF & β-TCP group at lingual side.

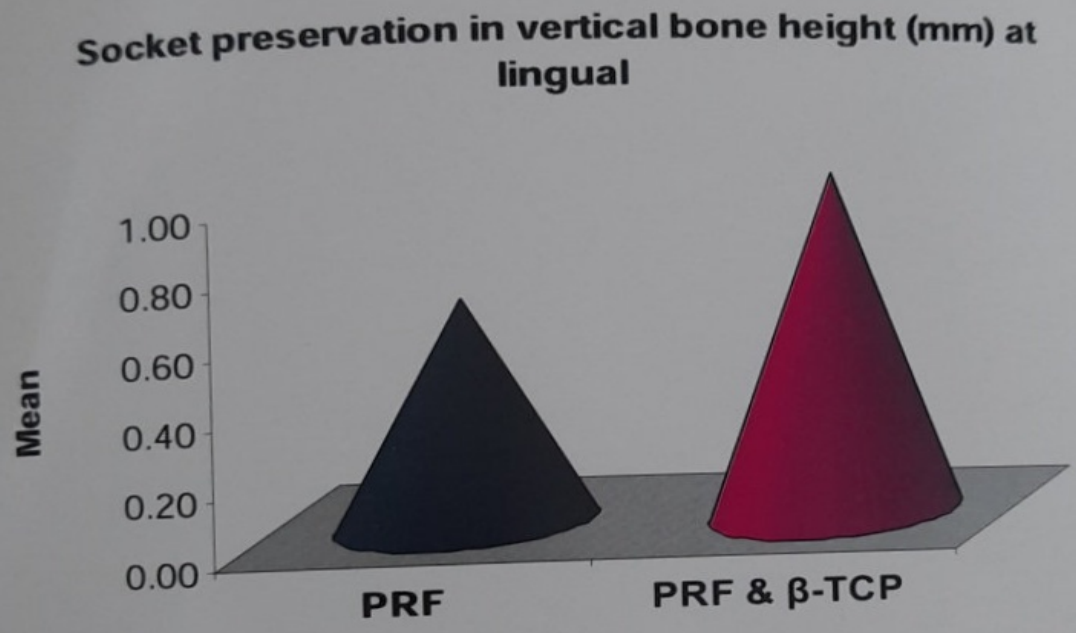


Fig. 7. Socket preservation in vertical bone height of two groups at lingual side.

B. Axial bone width

I. At crest (i.e 0 mm)

The pre and post axial bone width of two groups (PRF and PRF & β-TCP) at 0 mm(at crest) are summarized in Table 4. The pretreatment mean (± SD) axial bone width of Group A at 0 mm was 10.31 ± 1.75 mm while at post it was 9.58 ± 1.76 mm. Similarly, in Group B, it was 11.72 ± 1.48 mm at pre and 11.35 ± 1.30 mm at post.

Comparing the pre and post mean axial bone width of Group A, paired t test revealed decrease (0.73±0.51 mm) in axial bone width at post as compared to pre (10.31 ±1.75 vs. 9.58 ± 1.76, t=4.55, p=0.001) (Table 4 and Fig. 8).

Similarly, comparing the comparing the pre and post mean axial bone width of Group B, paired t test showed significant decrease (0.37 ± 0.49mm) in axial bone width at post as compared to pre (11.72± 1.48 vs. 11.35 ± 1.30, t=2.40, p=0.040) (Table 4 and Fig. 9).

Further, comparing the mean socket preservation (i.e. mean change from pre to post) in axial bone width at 0 mm of two groups, Student's t test showed socket preservation in axial bone width between the two groups (0.37 ± 0.49 vs. 0.73 ± 0.51, t=1.62, p=0.123) in which Group B is highly significant for preservation of socket which was 49.3% higher than Group A. (Table 4 and Fig. 10).

Table 4: Pre and post axial bone width (Mean ± SD) of two groups at 0 mm

Groups	Pre (n=10)	Post (n=10)	Change (Pre-Post)	t value	p value
A	10.31±1.75	9.58±1.76	0.73±0.51	4.55	0.001
B	11.72 ± 1.48	11.35±1.30	0.37 ± 0.49	2.40	0.040

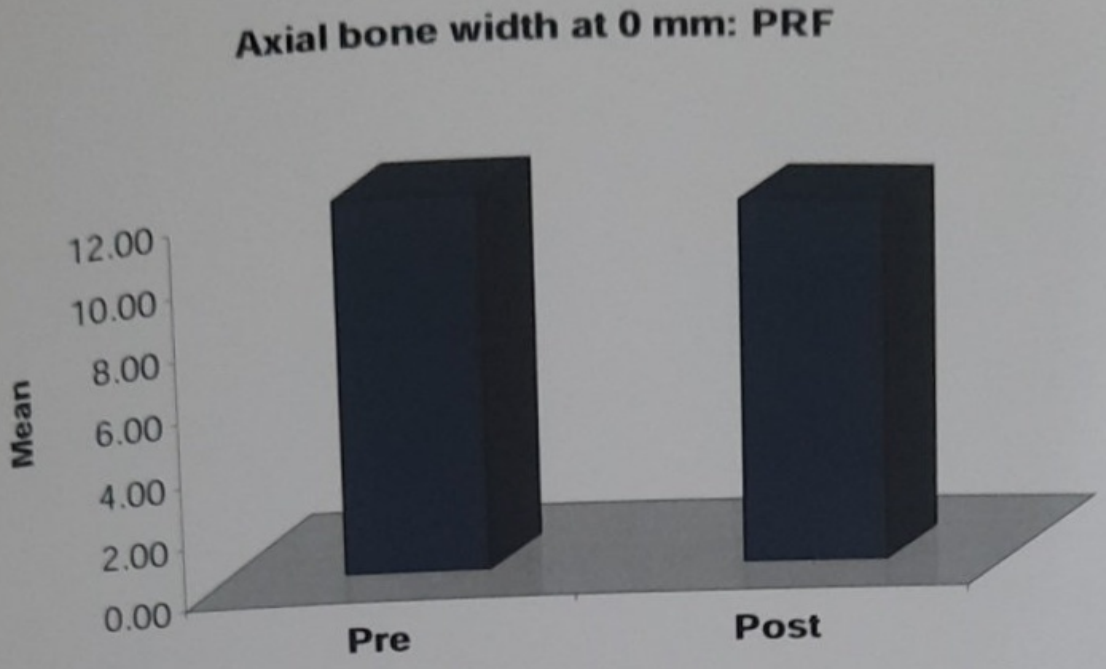


Fig. 8. Pre and post axial bone width of PRF group at 0 mm.

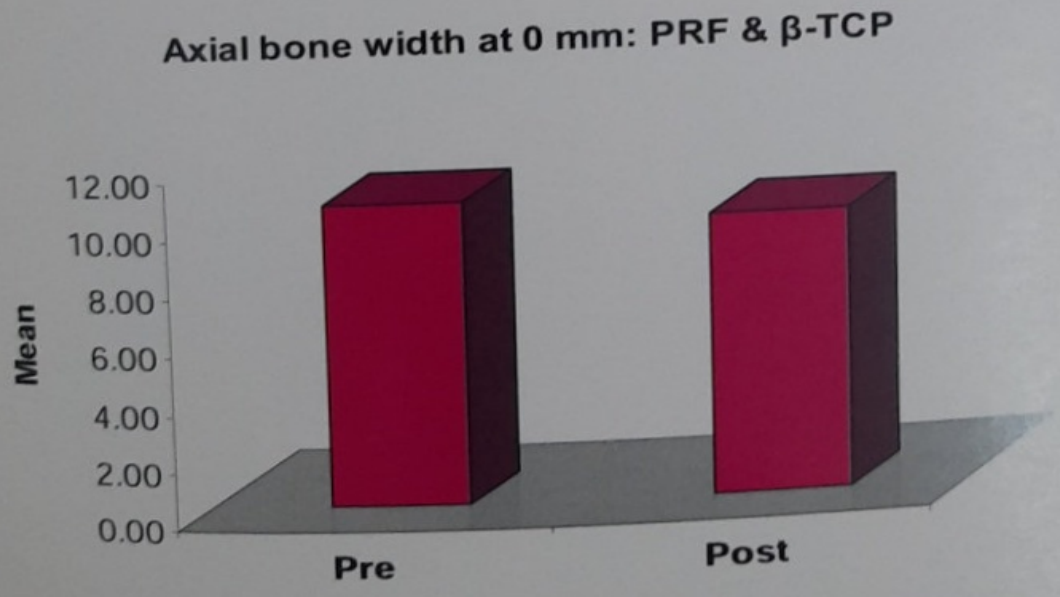


Fig. 9. Pre and post axial bone width of PRF & β-TCP group at 0 mm.

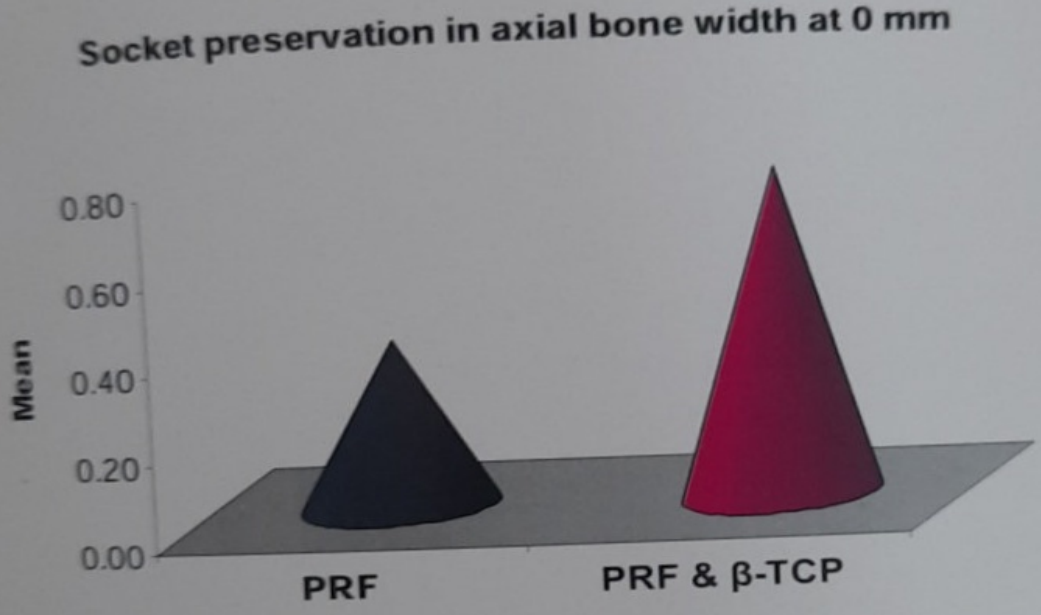


Fig. 10. Socket preservation in axial bone width of two groups at 0 mm.

II. 1 mm(from the crest)

The pre and post axial bone width of two groups (A and B) at 1 mm are summarized in Table 5. The pretreatment mean (± SD) axial bone width of Group A at 1 mm was 12.48 ± 1.70 mm while at post it was 11.55 ± 1.70 mm. Similarly, in Group B, it was 13.09 ± 1.53 mm at pre and 12.67 ± 1.24 mm at post.

Comparing the pre and post mean axial bone width of PRF group, paired t test revealed significant decrease (0.93 ± 0.95 mm) in axial bone width at post as compared to pre (12.48 ± 1.70 vs. 11.55 ± 1.70, t=3.11, p=0.013) (Table 5 and Fig. 11).

Similarly, comparing the comparing the pre and post mean axial bone width of Group B, paired t test showed significant decrease (0.42 ± 0.63 mm) in axial bone width at post as compared to pre (13.09 ± 1.53 vs. 12.67 ± 1.24, t=2.10, p=0.066) (Table 5 and Fig. 12).

Further, comparing the mean socket preservation (i.e. mean change from pre to post) in axial bone width at 1 mm of two groups, Student's t test showed socket preservation in axial bone width between the two groups (0.93 ± 0.95 vs. 0.42 ± 0.63, t=1.42, p=0.174) in which Group B is highly significant for preservation of socket which was 54.8% higher than Group A. (Table 5 and Fig. 13).

Table 5: Pre and post axial bone width (Mean ± SD) of two groups at 1 mm

Groups	Pre (n=10)	Post (n=10)	Change (Pre-Post)	t value	p value
A	12.48 ± 1.70	11.55 ± 1.70	0.93 ± 0.95	3.11	0.013
B	13.09±1.53	12.67±1.24	0.42±0.63	2.10	0.066

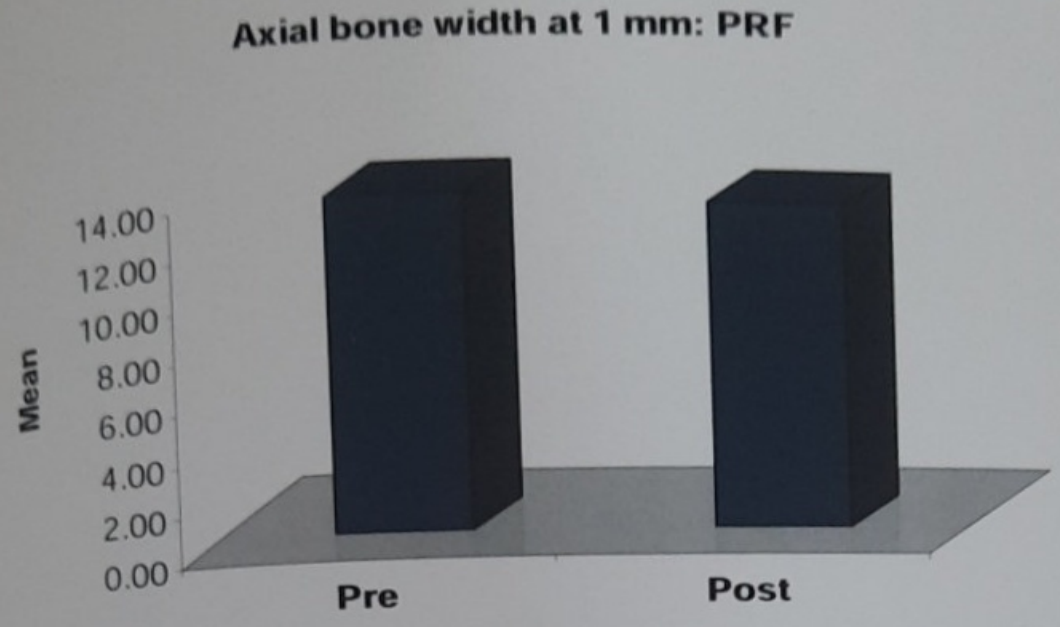


Fig. 11. Pre and post axial bone width of PRF group at 1 mm.

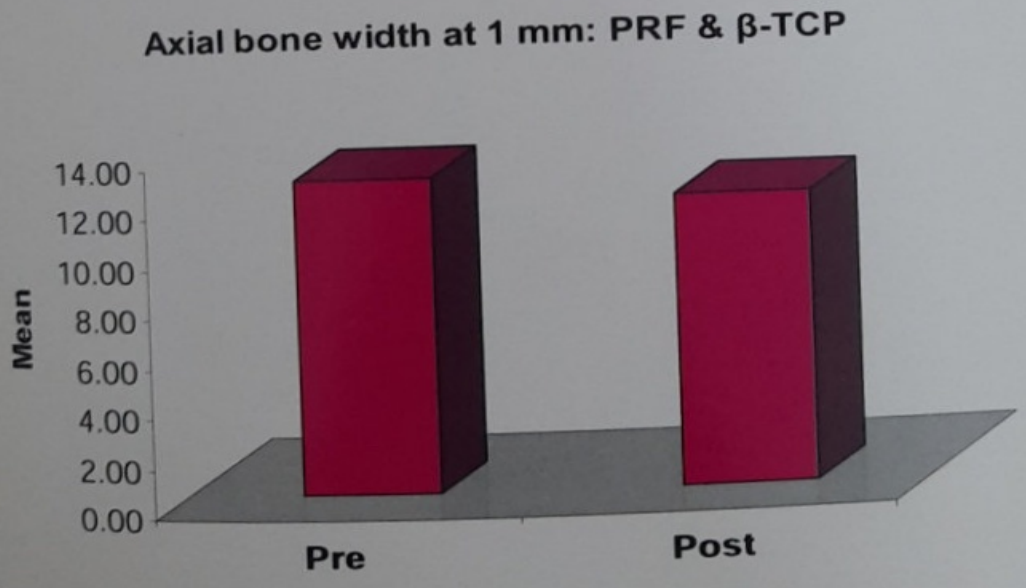


Fig. 12. Pre and post axial bone width of PRF & β-TCP group at 1 mm.

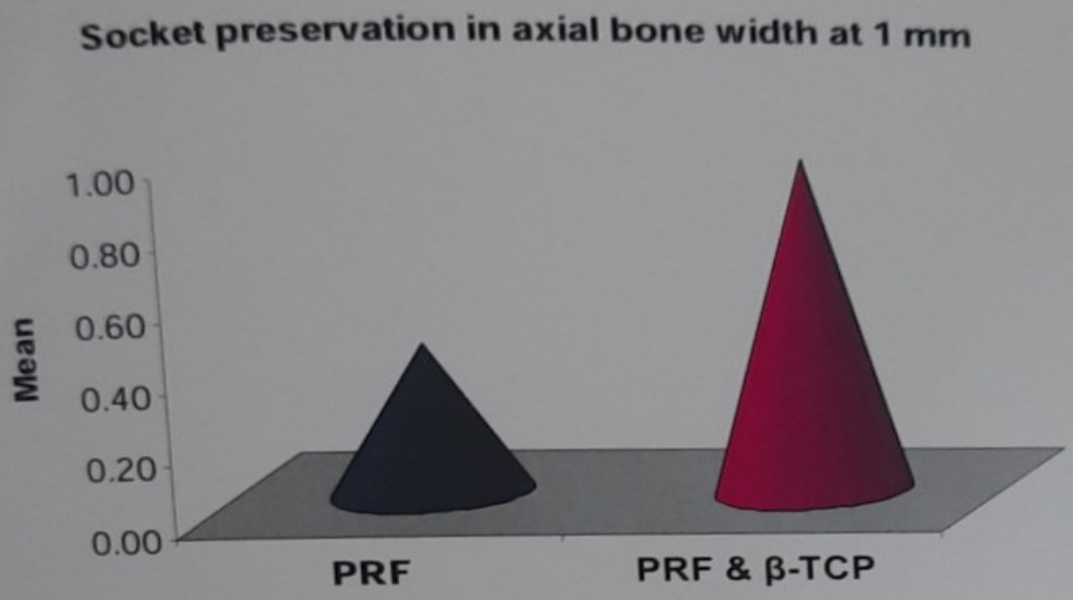


Fig. 13. Socket preservation in axial bone width of two groups at 1 mm.

III. 5 mm(from the crest)

The pre and post axial bone width of two groups (A and B) at 5 mm are summarized in Table 6. The pretreatment mean (± SD) axial bone width of Group A at 5 mm was 13.87 ± 1.43 mm while at post it was 12.96 ± 1.44 mm. Similarly, in Group B, it was 14.30 ± 1.29 mm at pre and 14.02 ± 1.15 mm at post.

Comparing the pre and post mean axial bone width of Group A, paired t test revealed significant decrease (0.91 ± 0.82 mm) in axial bone width at post as compared to pre (13.87± 1.43 vs. 12.96 ± 1.44, t=3.52, p=0.007) (Table 6 and Fig. 14).

Similarly, comparing the comparing the pre and post mean axial bone width of Group B, paired t test showed significant decrease (0.28 ± 0.55 mm) in axial bone width at post as compared to pre (14.30 ± 1.29 vs. 14.02 ± 1.15, t=1.60, p=0.144) (Table 6 and Fig. 15).

Further, comparing the mean socket preservation (i.e. mean change from pre to post) in axial bone width at 5 mm of two groups, Student's t test showed socket preservation in axial bone width between the two groups (0.91 ± 0.82 vs. 0.28 ± 0.55, t=2.02, p=0.059) in which Group B is highly significant for preservation of socket which was 69.2% higher than Group A (Table 6 and Fig. 16).

Table 6: Pre and post axial bone width (Mean ± SD) of two groups at 5 mm

Groups	Pre (n=10)	Post (n=10)	Change (Pre-Post)	t value	p value
A	13.87 ± 1.43	12.96 ± 1.44	0.91 ± 0.82	3.52	0.007
B	14.30±1.29	14.02±1.15	0.28±0.55	1.60	0.144

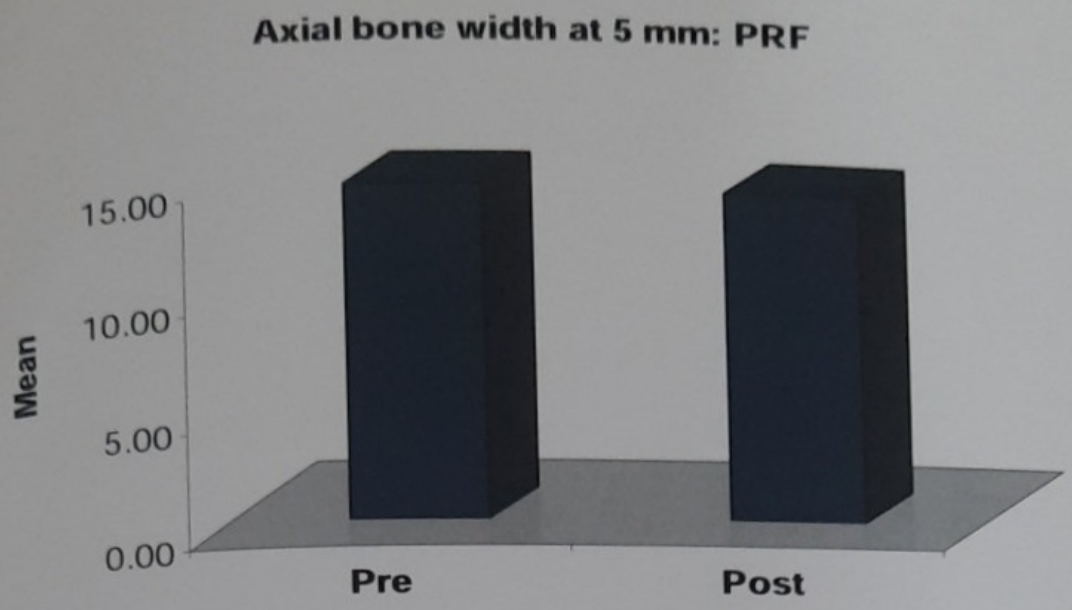


Fig. 14. Pre and post axial bone width of PRF group at 5 mm.

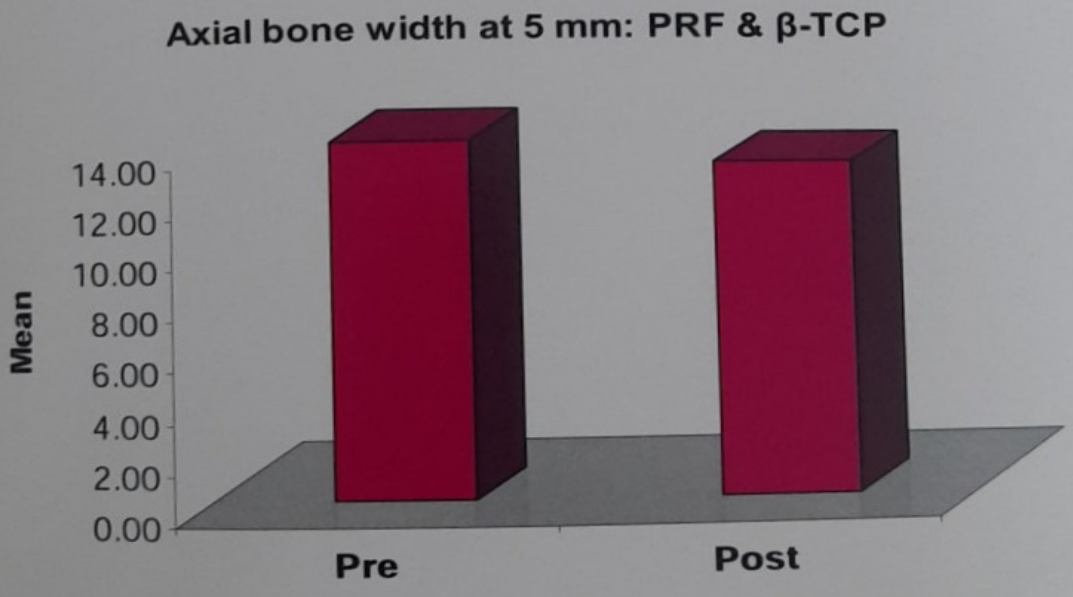


Fig. 15. Pre and post axial bone width of PRF & β-TCP group at 5 mm.

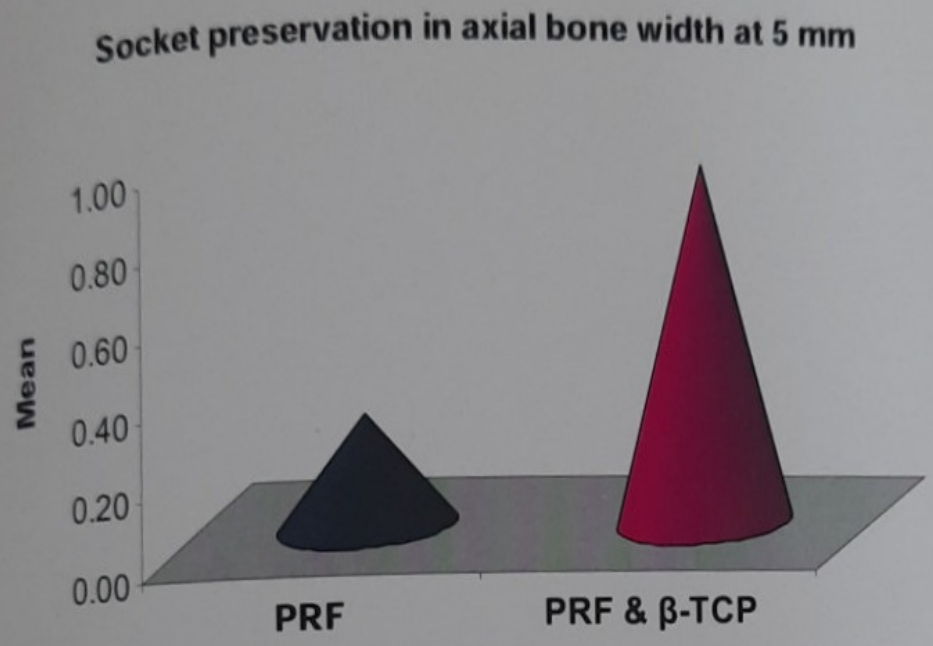


Fig. 16. Socket preservation in axial bone width of two groups at 5 mm.

Discussion

The healing of an extraction socket is characterized by both internal and external changes that ultimately affect the shape of the alveolar ridge[45]. By maintaining the original ridge morphology, there will be a minimal need for future augmentation procedures thereby allowing the resultant prosthetic implant restoration to be placed in an aesthetically and functionally ideal position[46].

During the last decade several different Alveolar Ridge Preservation (ARP) techniques have been developed, most of which include the use of a graft material that is placed into the extraction socket. Autogenous bone graft, which has favourable osteogenic, osteoinductive and osteoconductive properties, remains the gold standard as bone grafting materials[47]. But they require the patient to undergo a second surgical (donor-site) procedure, which increases patient morbidity. Other bone graft like allogeneic bone grafts have important advantages, such as unlimited quantity and a variety of forms (e.g., putty, particulate, gel), but have some disadvantages, including the potential for transmission of disease, immune reaction, increased treatment cost, and non osteoinductive properties[48].

Characteristics for ideal bone replacement graft materials include safety, efficacy, and the ability for the graft material to be replaced by vital alveolar bone. The β-Tricalcium Phosphate graft material used in this study is a purely synthetic alloplastic material fabricated to exact chemical specifications. As per the manufacturer's claim the porosity (of the β-TCP materials), both macroscopic and microscopic, is designed to maximize blood clot stability during early healing[50]. The material has no organic

components and, therefore, no chance of antigenicity or allergic reactions. As the material is synthesized in the laboratory, any possible infectivity is removed. There have been recent concerns regarding possible disease transmission from allografts and xenografts[51]. Using an alloplastic regenerative material prevents this possibility, eliminating the surgeon's as well as the patient's apprehension. Because of the chemical composition and high purity of the material, Tadic D (2004) have shown that no cytotoxic compounds are released during breakdown and resorption of this graft material[49].

Recently Platelet-rich fibrin (PRF) which was taken as a graft material for ARP, was first described by Choukroun as cited by Dohan et al in 2006[53]. It is considered a second-generation platelet concentrate and has been used in various surgical procedures in an attempt to enhance wound healing. It is prepared from the patient's own blood thereby eliminating the possibility of disease transmission or foreign body reactions similar to any autogenous graft[50]. The preparation technique of PRF is simple and requires no special equipment which minimizes cost of the treatment. Blood is drawn into standard glass blood collection tubes and centrifuged at a 3000 rpm to ensure cell separation. No anticoagulants are used during the procedure and natural coagulation can therefore take place. This unique preparation technique allows PRF to trap at least 95% of the platelets of the collected blood into a fibrin mesh[55]. The fibrin mesh can then be easily manipulated into a membrane that allows it to be transferred to any surgical site. High concentrations of the collected platelets allow for the slow release of growth factors (GFs) from the platelet granules[51].

Present study was conducted to quantify the changes in alveolar ridge dimensions after using β- TCP and autologous PRF as a bone graft in two different groups. The results were evaluated on the basis of radiological parameters, measured over conventional CBCT at pre treatment (at base line) and post treatment (after 4 month).

Vertical bone height:

At the buccal aspect there was (0.48 ±0.37) gain in vertical bone height in the GroupA and (0.90 ±0.46) increase in the Group B. When mean changes in vertical bone height at buccal aspect of two groups were compared, Group B was found to be 46.7% superior over Group A.

At lingual aspect there was (0.64 ±0.35) preservation in vertical bone height in the GroupA and (0.98 ±0.29) gain in Group B. When mean changes in vertical bone height at lingual aspect of two groups were compared, here also Group B was found to be 34.7% superior over Group A.

The finding at buccal and lingual aspect shows that in both the groups (Group A and Group B) there was a gain in vertical bone height. This means that there was no bone resorption of the extracted alveolous after graft placement, infact it showed a socket preservation. The difference in preservation of socket between the two groups,it shows that Group B was more effective for alveolar ridge preservation technique.

Bucco- lingual width (Axial cross-section):

Decrease in the bucco-lingual measurement in axial cross-section indicated actual decrease in bone width. At crest of the ridge (0mm) there was (0.73 ±0.59) decrease in bucco-lingual width in Group A and (0.37 ±0.49) decrease in Group B. When mean changes in bucco-lingual width at crest of the ridge between the two groups were compared, In Group B, preservation in the width was 49.3% higher than Group A.

At 1mm apical to crest of the ridge there was a decrease in bone width, Group A shows (0.93±0.95) decrease in bone width and Group B shows (0.42±0.63) when mean changes in bucco-lingual width at 1mm of crest of the ridge of two groups were compared, Group B preservation in the width was 54.8% higher than Group A.

At 5mm apical to crest of the ridge there was decrease in bone width. Group A shows (0.91±0.82) decrease in bone width and Group B shows (0.28±0.55) decrease in bone width. Here also when mean changes in bucco-lingual width at 5mm of crest of the ridge of two groups were compared, Group B preservation in the bone width was 69.2% higher than Group A.

These results at 0mm, 1mm and 5mm apical to the crest of the ridge shows that there is gain in bone width in both the groups but Group B is more effective in ridge preservation when compare with Group A.

Studies conducted in past by various authors such as Pietrokovski et al (2007) etc have shown that the socket without graft has significant resorption rate in both

vertical as well as bucco-lingual dimensions[52]. Here in our study no resorption is reported in the vertical bone height, on the other part a preservation was obtained if we compare our study with the studies without graft. Brkovik BM et al (2008) conducted study with β- TCP (RTR Cone, Septodont) and measured its efficacy in alveolar ridge preservation (ARP) on the day of implant placement revealed slight horizontal bone resorption, but no change in the vertical dimension of the alveolar ridge[53]. Our study is in accordance to them where we also observed similar trend with β- TCP.

Iasella JM et al[60] (2003) conducted study with freeze-dried bone allograft and a collagen membrane for ARP and concluded that no treatment of the socket resulted in a collapse of the alveolus by 29% and grafting the socket at the time of extraction resulted in bone loss of only 13%[54]. Araújo MG et al (2014) conducted sudy with a Bio-Oss Collagen (xenograft) and concluded that cross-sectional area of the control ridge was reduced about 25% and of the test group (Bio-Oss) with 3%.whereas in this study there was gain in vertical bone height of 2.8% with PRF and 6.3% gain with β- TCP. By this observation it can be inferred that both β- TCP an alloplast material as well as PRF an autograft are superior to many allograft and xeno graft used till now[55].

Suttapreyasri S & Leepong N (2013) conducted study to investigate the influence of platelet-rich fibrin (PRF) on early wound healing and preservation of the alveolar ridge shape following tooth extraction[56]. Mazor et al. successfully used PRF as the

only grafting material in a series of sinus augmentation procedures. With this technique they were able to demonstrate that PRF could stimulate new bone formation in areas that were previously deficient of the amount of bone required for implant placement. Toma et al 2015[57] In vitro studies have confirmed that PRF selectively stimulates the growth of osteoblasts and gingival cells. So the gain in bone height seen in the present study exclusively with PRF can be atrributed to these above reasons.

Pradeep AR and Sharma A (2011) have shown that PRF could be used as a guided tissue- regeneration (GTR) membrane to affect periodontal regeneration in 3-wall bony defects and degree II furcation lesions[58]. This property of PRF eliminates use of any commercial membrane making it further cost effective.

In a study conducted by Simon et al 2009, it was observed that when membrane was used with PRF or DFDBA, results were better in PRF alone group where membrane was not used showing the disadvantages of using barrier membrane. So in PRF group we did not placed any membrane to get better results. β- TCP graft could not be placed without barrier membrane as chances of dislodgement of the particles was always there[59].

In 1995 Fuhrmann R in his study used helical CT for the identification of buccal and lingual alveolar bone. Only alveolar bone plates with the thickness smaller than 0.2 mm could not be apparent in medical CT images[60]. Moreover, a study in human cadavers showed that artificial horizontal bone defects made in the buccal and lingual

alveolar plates were identified in helical CT images while could not be visualized in periapical radiographs. In 1996, an experimental study which performed artificial bone dehiscences in the maxillary bone of human cadavers has concluded that CT was the only mean of diagnosis which permits a quantitative evaluation of buccal-lingual thickness of both the alveolar ridge and the buccal and lingual bone plates[65]. So in this present study for the accurate measurement of buccal/lingual cortical bone height and bucco-lingual bone width, CBCT of jaw was done, with the help of inbuilt software 3D images were produced for result analysis more accuracy So CBCT was utilized to get conclusive results with minimal calibrating errors.

Dincer Yilmaz et al (2014) conducted study where they prepared four standardized defects in both tibias of three adult male pigs. The first defect was left unfilled as a control, the others were grafted with either PRF, β-TCP and PRF mixed with β-TCP[61]. Histological study reveals more new bone formation, including osteoblasts and osteocytes in the connective tissue, in combination group. It is thought that PRF accelerates the healing effect by keeping the particles of β-TCP together via its adhesive property and adapting them tightly to the walls of the cavity[62].

A study was conducted in 2004 by Wiltfang et al. with PRP in combination with β–TCP where they found β–TCP particles remaining in the bone defects after 12 weeks[63].

Studies have shown that PRF contains various growth factors which includes vascular endothelium growth factor (VEGF), platelet-derived growth factor (PDGF),

fibroblast growth factor (FGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF)[64], insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), and transforming growth factor-beta (TGF-beta). All of these play a role in replacing lost tissue, resurfacing of the wound, and restoring vascular integrity[65]. Compared to other platelet concentrates, PRF releases these factors at a sustained rate over a longer period, thereby optimising wound healing. Tomar et al 2015[66] *In vitro* studies have confirmed that PRF selectively stimulates the growth of osteoblasts and gingival cells. So the gain in bone height seen in this present study exclusively with PRF can be attributed to these above reasons[67]

Bhatt AK et al in 2015[68] conducted a study in which they compared between PRF alone and β-TCP alone and found that β-TCP alone was slightly but insignificantly superior to PRF alone. So, in continuation of this study and the current study of ours was under taken to compare combination of PRF with β-TCP with PRF alone.

Since, PRF is cost- effective, easy to prepare and it is one of the best autologus graft for combination with any alloplastic graft for the preservation of alveolar ridge. Therefore it could cut the cost to half for the needy patients in our study.

Conclusion

Analysis of results obtained from preoperative and postoperative CBCT of alveolar ridge are concluded here:

- The Group A showed a 2.8% gain in vertical height at buccal aspect and 1.5% gain in vertical height at lingual aspect of the extracted alveolar socket.
- Group B showed a 6.3% gain in vertical height at buccal aspect and 3.7% gain in vertical height at lingual aspect of the extracted alveolar socket.
- The gain in vertical height was 50.8% and 46.2% more at buccal and lingual aspect respectively with Group B over Group A. These results indicates that combination of PRF and β-TCP is significantly better than PRF alone for vertical bone gain.
- In contrary to gain in bone width there was slight reduction of 2.6% at 0mm, 3.1% at 1mm and 1.6% at 5mm from the crest buccolingually with Group B.
- Similarly there was slight reduction of 2.4% at 0mm, 1.6% at 1mm and 0.7% at 5mm from the crest buccolingually with Group A..
- The decrease in bone width at crest (0 mm) is 49.3% less, at 1mm is 54.8% less, and at 5mm is 69.2% less in Group B over Group A. These results also indicates that combination of PRF and β-TCP is significantly better than PRF alone for bone width dimensions.

From the above result it can be said that PRF in combination with β–TCP is more effective when compared with PRF alone as a graft material in alveolar ridge preservation technique.

Since PRF is an economical as well as autologous material without any chemical modification and is also easy to prepare, it would be the first choice for ARP, in combination with any other alloplastic graft. Future studies can always be conducted with larger sample size to support these results.

Summary

Periodontal diseases and dental caries when remains untreated, lead to progressive destruction and loss of either supporting periodontium or loss of tooth structure such teeth with hopeless prognosis are indicated for extraction.

Tooth extraction is usually followed by partial resorption of the residual alveolar ridge, approximately 0.34 to 7.7 mm of horizontal ridge reduction and 0.2 and 3.25 mm of vertical ridge reduction in the 6 to 12 months period following extraction. If socket remains untreated, the resulting deformity can be a considerable obstacle to the esthetic, phonetic, and functional aspect for both patients and clinicians.

With the aim of minimizing the need for future tissue augmentation, several different alveolar ridge preservation techniques are proposed, most of which include the use of a graft materials which can be autogenic, allogeneic, xenogeneic or synthetic bone graft material, or a combination of these products. Beta-tricalcium phosphate (β-TCP), a synthetic alloplastic material which is resorbable and have osteoconductive property, has been used for bone regeneration in a variety of surgical procedures with satisfactory clinical and histologic results.

Lost hard or soft-tissue structures can also be regained when periodontal tissues are provided an appropriate environment during healing phase after surgical therapy. Choukroun introduced platelet rich fibrin (PRF), a second-generation platelet concentrate that improves healing of the both hard and soft tissues and can be used as alternative to bone grafts. PRF is autologous without any artificial biochemical

modification, has no antigenic property and forms a strong natural fibrin matrix, which concentrates almost all the platelets and growth factors of the blood harvest.

A 4- month clinical study was carried out with 20 patients irrespective of gender, suffering from chronic periodontitis with grade-III mobility or having grossly decayed non restorable tooth. Aim was to evaluate the effectiveness and clinical outcome of Platelet Rich Fibrin (PRF) and combination of PRF with β- Tricalcium phosphate as graft material for alveolar ridge preservation in extraction socket. For radiographic analysis a CBCT of jaw was done. Any reduction in this measurement from pre treatment to post treatment indicates gain in vertical bone height. Bucco-lingual/palatal width of alveolar bone : It was measured at mid crest region in a axial view at crest of the ridge 0, 1 and 5 mm apical to the crest recorded to the nearest millimeter. Any reduction in this measurement from pre treatment to post treatment indicated actual loss of alveolar ridge width.

After phase-I therapy in all the patients, surgical therapy was carried out as per the inclusion and exclusion criteria. In PRF group prior extracrtion PRF preparation was done. Atraumatic extraction was done and PRF graft was placed without any membrane. In PRF with β-TCP group before extraction full thickness flap was raised up to mucogingival junction to facilitate graft coverage and resorbable collagen membrane was used between flap and graft. Sutures and coe pack was given for two weeks. The results were evaluated on the basis of radiological parameters, measured

over conventional CBCT at pre treatment (at base line) and post treatment (after 4 month).

Vertical bone height: At the buccal aspect there was 2.8% (0.38 ±0.45) increase in vertical bone height in the PRF group and 6.3% (0.76 ±1.28) increase in the PRF with β- TCP group. When mean changes in vertical bone height at buccal aspect of two groups were compared,PRF with β- TCP group was found to be 50.8% superior over PRF group.

At lingual aspect there was 1.5% (0.18 ±0.92) increase in vertical bone height in the PRF group and 3.7% (0.33 ±1.01) increase in the PRF with β- TCP group. When mean changes in vertical bone height at lingual aspect of two groups were compared, PRF with β- TCP group was found to be 46.2% superior over PRF group.

The finding at buccal and lingual aspect shows that in both the groups (PRF and PRF with β-TCP) there was gain in vertical bone height. This means that there was no bone resorption of the extracted alveolous after graft placement, infact it showed a gain..

Bucco- lingual width (Axial cross-section): Decrease in the bucco-lingual measurement in axial cross-section indicated actual decrease in bone width. At crest of the ridge (0mm) there was 2.6% (0.31 ±0.58) decrease in bucco-lingual width in PRF group and 2.4% (0.25 ±0.55) decrease in the PRF with β- TCP group. When mean changes in bucco-lingual width at crest of the ridge between the two groups

were compared, In PRF group decrease in the width was 20.0% higher than PRF with β- TCP group. At 1mm apical to crest of the ridge there was 3.1% (0.41 ±0.71) decrease in PRF group and 1.6% (0.19 ±0.39) decrease in the β- TCP group. When mean changes in bucco-lingual width at 1mm of crest of the ridge of two groups were compared, in PRF group decrease in the width was 54.5% higher than β- TCP group. Although student's t test reveals this difference as non significant. At 5mm apical to crest of the ridge there was 1.6% (0.23 ±0.64) decrease in PRF group and 0.7% (0.09 ±0.26) decrease in the β- TCP group. Here also when mean changes in bucco-lingual width at 5mm of crest of the ridge of two groups were compared, In PRF group decrease in the width was 61.1% higher than β- TCP group. Although once again student's t test reveals this difference as non significant. These results at 0mm, 1mm and 5mm apical to the crest of the ridge shows that there was bucco- lingual resorption but it was statistically insignificant.

Both the materials β- TCP and PRF are able to preserve the bucco-lingual width of the socket up to some extent. Since PRF being autologous, without any chemical modification, easy to prepare and eliminates the use of any barrier membrane or mucoperiosteal flap surgery to cover the PRF graft, ARP proceduce becomes simplified. Being economical, it can be first choice as a graft in Indian scenario and PRF with Beta tri-calcium phosphate is good choice for preservation of alveolar socket.

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Appendices

APPENDIX – I

Babu Banarasi Das College of Dental Sciences
(A Faculty of Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow – 227105 (INDIA)

Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the 3rd Institutional Ethics Sub Committee Meeting.

BBDCODS/ 29 /2015

IEC Code: 29

Title of the Project: Comparative Evaluation Of Autograft V/S Allograft For Alveolar Ridge Preservation- A Clinical Radiographic Study.

Principal Investigator: Dr. Kumar Shantanu **Department:** Periodontology

Name and Address of the Institution: BBD College of Dental Sciences Lucknow

Type of Submission: New, MDS Protocol

Dear Dr. Kumar Shantanu

The Institutional Ethics Sub Committee meeting was held on 09-01-2015. The sub committee comprises following four members :

1.	Dr. Amrit Tandon Member	Prof. & Head, Deptt. of Prosthodontics BBDCODS, Lucknow.
2.	Dr. Jiji George Member	Prof., Deptt. of Oral Pathology & Microbiology, BBDCODS, Lucknow.
3.	Dr. Ashish Saini Member	Reader, Department of Periodontology, BBDCODS, Lucknow.
4.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Deptt. of Biochemistry, BBDCODS, Lucknow.

The committee reviewed and discussed your submitted documents of the research study in the meeting. The proposal was reviewed and thoroughly revised.

Decisions of the IEC : As per the recommendations I.E.C. has taken following decisions for the current protocol of study "Comparative Evaluation Of Autograft V/S Allograft For Alveolar Ridge Preservation- A Clinical Radiographic Study."

The committee approved the above proposal from ethics point of view.

Lakshmi Bala 19/1/15

(Dr. Lakshmi Bala)
Member-Secretary IEC

Member-Secretary
Institutional Ethics Committee
Faculty of Dental Sciences
BBD University
Lucknow-226028

Forwarded by:

(Dr. Vivek Govila)
Dean

DEAN
BBD College of Dental Sciences
BBD University
Faizabad Road Lucknow-226028

APPENDIX - II

CASE SHEET

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES, LUCKNOW

"A Comapative Evaluation Of PRF With PRF with beta tri-calcium phosphate For Alveolar Ridge Preservation In Extraction Socket- ***A Clinico- Radiographic Study"***

CLINICAL EVALUATION:

NAME: **AGE:** **SEX:**

DATE:

OPD No.:

ADDRESS:

Chief complaint:

History of present illness:

GROUPS:

GROUP A	PRF Group
GROUP B	PRF with β-TCP group

Past dental history:

Past medical history:

History of medication:

Hard tissue examination:

1. Buccal and Lingual vertical bone height at baseline and at 4 months Post-Operative.

2.Horizontal bone width (at baseline)

i) At 0 mm (at the crest):

ii) At 1mm (from the crest):

iii) At 5 mm (from the crest):

3.Horizontal bone width (at 4 month postoperative)

i) At 0 mm (at the crest):

ii) At 1mm (from the crest):

iii) At 5 mm (from the crest):

CLINICAL EVALUATION:

I. Gingiva

Color

Consistency

Size

Position

Bleeding

Suppuration

II. Examination of teeth:

Number of teeth present

Mobility

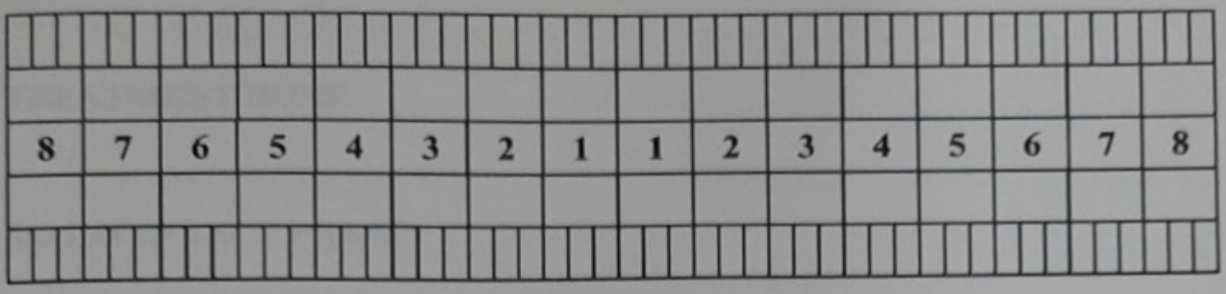
CLINICAL PERIODONTAL PARAMETERS

PLAQUE INDEX (Silness & Loe)

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

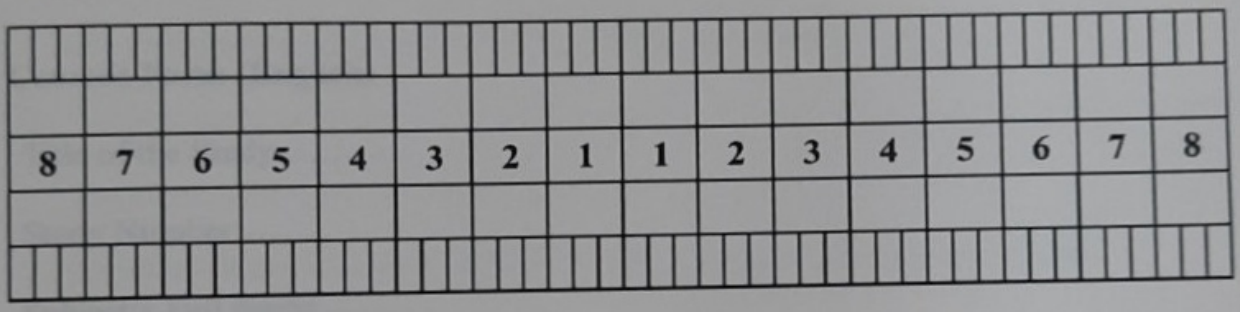
PLAQUE SCORE =

GINGIVAL INDEX (Loe & Silness)

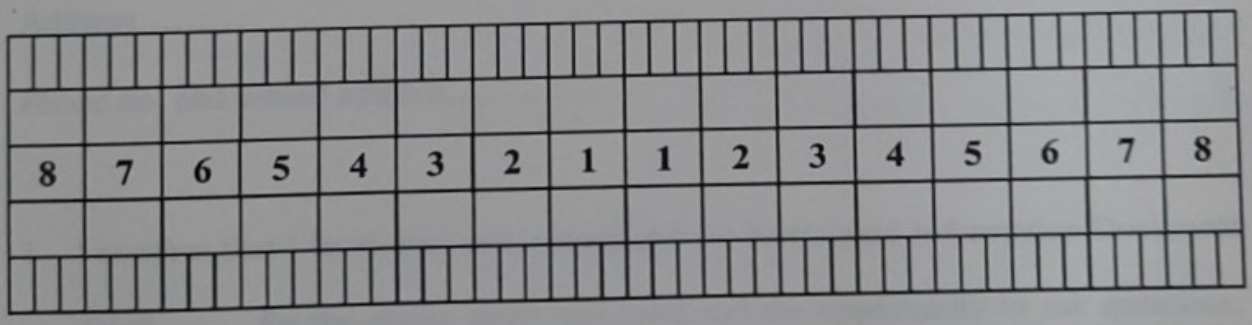


GINGIVAL SCORE =

PROBING POCKET DEPTH



CLINCAL ATTACHMENT LEVEL



DIAGNOSIS

PROGNOSIS

TREATMENT PLAN

TREATMENT DONE

MAINTENANCE PHASE

APPENDIX - III

Consent Form (English)

Title of the Study .....

Study Number.....

Subject's Full Name.....

Date of Birth/Age.....

Address.....

Phone no. and e-mail address.....

1. I confirm that I have read and understood the Participant Information Document dated .....for the above study and have had the opportunity to ask questions. **OR** I have been explained the nature of the study by the Investigator and had the opportunity to ask questions.
2. I understand that my participation in the study is voluntary and that I am free to

withdraw at any time, without giving any reason and without my medical care or legal rights being affected.

3. I understand that the sponsor of the project, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. However, I understand that my Identity will not be revealed in any information released to third parties or published.
4. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).
5. I permit the use of stored sample (tooth/tissue/blood) for future research.

   Yes [ ] No [ ] Not Applicable [ ]
6. I agree to participate in the above study. I have been explained about the complications and side effects, if any, and have fully understood them. I have also read and understood the participant/volunteer's Information document given to me.

Signature (or Thumb impression) of the Subject/Legally Acceptable Representative:.....

Signatory's Name..... Date .....

Signature of the Investigator..... Date.....

Study Investigator's Name..... Date.....

Signature of the witness..... Date.....

Name of the witness.....

Received a signed copy of the PID and consent form

Signature/thumb impression of the subject or legally acceptable representative Date.....

APPENDIX-IV

POST OPERATIVE INSTRUCTIONS AFTER TOOTH EXTRACTION

- After leaving the clinic take ivities for the remainder of the day.
- Do not touch extraction site with ton
- Take proper medication as prescribed.
- Do not spit or rinse mouth vigorously.
- Avoid hot food and drinks.
- Take cold food and cold beverages but avoid soft drinks (aerated).
- Do not brush your teeth for the 8 hours after surgery.
- After 24hrs of extraction, warm saline rinses, 2-3 times a day for next 5 days should be do

APPENDIX-V

STATISTICAL TOOLS EMPLOYED

Formula used for the analysis

The Arithmetic Mean

The most widely used measure of central tendency is arithmetic mean, usually referred to simply as the mean, calculated as

$$\bar{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

The Standard Deviation

The standard deviation (SD) is the positive square root of the variance, and calculated as

$$SD = \sqrt{\frac{\sum X_i^2 - \frac{(\sum X_i)^2}{n}}{n-1}}$$

where, n= no. of observations

Paired t-test

Paired t-test was used to calculate the differences between two paired samples i.e. when in each observation in Sample 1 is in some way correlated with an observation in Sample 2, so that the data may be said to occur in pairs and calculated as

$t = d/S_d$

where, d is the mean of difference within each pair of measurements and S_d the standard error of the difference. The degrees of freedom (DF) is calculated as

$DF = n\text{-}1$

Student's t-test

Student's t-test was used to calculate the differences between the means of two groups

$$t = \frac{\bar{X}_1 - \bar{X}_2}{SE}$$

$$SE = \sqrt{S^2 X \left(\frac{1}{n_1} + \frac{1}{n_2} \right)}$$

S^2 is the pooled variance and n_1 and n_2 are number of observations in group 1 and 2 respectively. The degrees of freedom (DF) is calculated as

$$DF = n1 + n2 - 2$$

Statistical significance

Level of significance "p" is the probability signifies level of significance. The mentioned p in the text indicates the following:

$p > 0.05$ Not significant (ns)

$p < 0.05$ Just significant (*)

$p < 0.01$ Moderate significant (**)

$p < 0.001$ Highly significant (***)

APPENDIX-VI

OBSERVATIONS

I. Vertical bone height (mm)

Subjects	PRF GROUP				PRF with β-TCP GROUP			
	Buccal		Lingual		Buccal		Lingual	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	2.4	2.2	10.4	10.0	4.2	3.6	1.4	1.1
2	15.4	14.1	19.2	17.4	15.4	14.9	8.7	8.9
3	21.8	21.1	0.1	1.7	8.1	7.9	5.4	5.4
4	10.3	10.1	11.5	11.2	18.3	14.4	14.4	11.6
5	17	16.5	15.2	14.8	7.1	6.8	7	6.9
6	9.1	8.9	12	11.7	19.1	18.9	15.9	15.8
7	17.9	17.8	12	11.8	13.4	13.1	8.7	8.4
8	12.6	12.5	11.5	11.4	11.3	11.2	8.8	8.8

II. Bucco-lingual (Axial) bone width (mm)

Subjects	PRF GROUP						PRF with β-TCP GROUP					
	At 0 mm		At 1 mm		At 5mm		At 0 mm		At 1 mm		At 5mm	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	10.2	10.1	12	11.8	14.9	15	12.4	12.7	13.4	13.5	15	14.8
2	13.8	13.4	15.7	14.8	16.5	16.5	9	7.5	9.4	9.1	12.1	11.5
3	9.8	9.7	11.8	11.4	12.9	12.9	9.9	9.5	11.7	11.6	13.8	13.5
4	13.6	11.9	15.2	13.2	15.6	13.8	9.4	9.3	11.3	11.3	13.5	13.5
5	12.4	12.3	13.1	13	15	14.9	8.7	8.5	10.7	10.7	10.9	10.9
6	12.6	12.4	14.4	14.3	12.7	12.7	13.4	13.2	15.5	14.4	13.4	13.4
7	12.4	12.6	13.2	13.1	14.5	14.5	9.5	9.4	10.6	10.6	13.7	13.2
8	10.8	10.7	11.3	11.2	13.9	13.9	12.4	11.9	13.4	13.3	14.8	14.6