Original Article

Evaluation of the relative efficacy of autologous platelet-rich fibrin membrane in combination with β-tricalcium phosphate (Septodont- resorbable tissue replacement)™ alloplast versus β-TCP alloplast alone in the treatment of grade II furcation defects

ABSTRACT

Introduction: Platelet-rich fibrin (PRF) is considered as the second-generation platelet concentrate, contains combined properties of fibrin, platelets, leukocytes, growth factors, and cytokines that make it as healing biomaterial with incredible potential for hard tissue and soft tissue regeneration. The present study was aimed to evaluate the effectiveness of PRF with β-tricalcium phosphate (β-TCP) graft (R. T. R) and compare it with β-TCP allograft alone in the treatment of mandibular Grade II furcation defects.

Material and Methods: A total of 20 mandibular Grade II furcation defects sites were assigned in the study and treated with either β-TCP alone (Group I) or β-TCP with PRF membrane (Group II). The clinical parameters analyzed were probing pocket depth (PPD), clinical attachment level (CAL), gingival recession (GR), horizontal defect depth (HDD), and vertical defect depth (VDD), recorded baseline and at 6 months reentry.

Results: At 6 months, both groups showed statistically significant results for all parameters from their baseline value, although intergroup changes were statistically insignificant. In Group I, gain in CAL was 2.80 ± 1.40 and in Group II it was 3.00 ± 1.44. Bone fill in Group I was VDD (3.50 ± 2.12) and HDD (3.70 ± 0.67), whereas Group II showed VDD (3.70 ± 1.57) and HDD (4.0 ± 0.88), respectively. PPD reduction was higher in Group I (3.50 ± 2.27) than Group II (2.80 ± 1.93). At reentry GR was established, Group I showed higher GR (0.70 ± 0.67) and Group II (0.40 ± 0.52).

Conclusions: Significant improvement was found in both groups, but the combination of PRF with β-TCP allograft led to more favorable improvement in the management of Grade II furcation defect except PPD.

Keywords: Furcation defects-Grade II, platelet-rich fibrin, regeneration, β-tricalcium phosphate allograft

INTRODUCTION

Chronic periodontitis is an inflammatory disease, cause destruction of periodontal tissues by disproportionate immunological responses to stimulating agents, such as pathogenic bacteria present in the dental biofilm.[1] Definite periodontal pathogens and associated host-mediated immune responses cause progressive loss of the tooth-supporting tissues which ultimately result in the formation of intraosseous defects or furcation defects. Furcation defect refers to the invasion of the bifurcation and trifurcation of

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multirooted teeth by periodontal disease, which offers a unique challenge for its treatment both from a prognostic perspective[2] and from the perspective of therapeutic measures.[3] The ideal goal of furcation therapy is to completely close the furcation defect and retain the tooth, thereby achieving the local condition to one of anatomic normalcy, facilitating long-term periodontal maintenance therapy, and the likelihood of tooth retention.

Although bone grafting methods[4,5] and the concept of tissue regeneration[6] has presented predictable treatment of furcation defects. Regeneration of alveolar bone, periodontal ligament and root cementum can be brought through the process of osteogenesis, osteoinduction and osteoconduction by the use of various bone graft (autogeneous, allogenic, or xenogenic bone graft). Inherent disadvantages of these bone graft materials include unpredictable resorption, additional surgical sites, time, and cost of the treatment.[7] To circumvent these demerits, synthetically produced bone substitute materials (i.e., alloplasts) have been used to eliminate the risk of disease transfer and procurement morbidity. The most widely used synthetic ceramic grafting materials are hydroxyapatite and beta-tricalcium phosphate. Among the variously available bone substitutes, beta-TCP is the one which is fully resorbed and replaced by natural local bone within a reasonable period. Furthermore, the use of b-TCP in humans have shown pocket depth reduction, gain in clinical attachment levels (CALs) and bone fill.[8,9]

Although these materials are still used today for reconstruction of the periodontium, some biological mediators such as enamel matrix derivatives, platelet-rich plasma (PRP), platelet-rich fibrin (PRF), platelet-derived growth factor, and bone morphogenetic proteins has opened new possibility in the treatment of furcation defects. Choukroun's PRF, a second-generation platelet concentrate, is an intimate congregation of cytokines, glycanic chains, and structural glycoproteins enmeshed within a fibrin network with synergetic effects on healing processes.[10-12] Favorable effects of PRF have been studied in various procedures, such as facial plastic surgery,[13] a sinus-lift procedure,[14] multiple gingival recession (GR) with a coronally displaced flap[15] and furcation defects and intrabony defect.[16,17]

Currently, the use of single regenerative material cannot be considered as the gold standard in the treatment of furcation defects. Considering that PRF along with bone graft may enhance the healing potential of both hard tissue and soft tissue, the present study was contemplated with the aims to evaluate the relative efficacy of autologous PRF membrane with β-tricalcium phosphate alloplast (septodont resorbable tissue replacement [RTR])™ versus β-tri-calcium phosphate (β-TCP) alloplast alone in the treatment of Grade II furcation defects.

MATERIALS AND METHODS

Study design
A total of 20 patients with chronic periodontitis, aged between 25 and 50 years, irrespective of their sex were selected from Outpatient Department of Periodontology, Faculty of Dental Sciences, King George's Medical University, Lucknow, Uttar Pradesh, India.

The inclusion criteria for the study included the presence of Grade II furcation defect with minimum 3 mm horizontal probing depth and good level of oral hygiene maintained after phase-I therapy.

Patients with a history of systemic illness, insufficient platelet count, pregnancy and/or lactation, a habit of using of tobacco products and smoking, allergy and those taking drugs known to interfere with wound healing were excluded from the study. In addition, teeth with inter-proximal intra-bony defects and endodontic involvement were also excluded from the study.

The patients were informed of the purpose and design of the study, and written consent was taken from each participant. Ethical clearance was obtained for the study from the ethical committee KGMU, Lucknow India.

Septodont resorbable tissue replacement™
RTR is a gradually resorbable material made of β-TCP granules of synthetic origin. It is osteoconductive microporous and macroporous structure that fosters dense new bone growth and available as granules, with size ranging between 500 μm and 1 mm. The size of macropores range from 100 μm to 400 μm and micropores are <10 μm in diameter which allows the colonization of macropores by newly formed bone.

Presurgical management
Each subject received Phase-I therapy which comprised full mouth supra and subgingival scaling and root planing with the provision of oral hygiene instructions. Before the surgical procedure, routine blood investigation was done in all the patients that came under the normal limits. Four weeks later, Phase-I therapy only before the surgical procedure, a periodontal evaluation was performed to confirm the suitability of sites for the study. A total of 20 otherwise healthy patients with furcation defects were randomly divided into two groups by a flip of coin method and having 10 patients in each group. Group I was treated with open flap debridement (OFD) followed by placement of β-TCP bone

graft and GroupII was treated with OFD and the placement of β-TCP bone graft together with autologous PRF as a barrier membrane.

The clinical parameters included soft tissue measurements as follows: probing pocket depth (PPD), CAL, GR, and hard tissue measurements: horizontal defect depth (HDD) and vertical defect depth (VDD) which was recorded at baseline and 6 months postsurgically using customized acrylic stents with grooves to ensure a reproducible placement of the calibrated University of North Carolina No. 15 (for vertical measurement). Horizontal furcation depth (distance from an imaginary line joining the maximum convexity of the mesial and distal roots) was measured with a 3 mm incrementally graduated Nabers's probe .

Platelet-rich fibrin membrane preparation

After the recipient site preparation was completed, 10 ml of blood was drawn in a 15 ml test tube without an anticoagulant and centrifuged immediately using a tabletop centrifuge (Systonic Lab and Scientific Instruments, INDIA) for 12 min at 3000 rpm. The resultant product consisted of following three layers: an uppermost layer of PPP (platelet poor plasma), PRF clot in the middle, RBC at the bottom. PRF clot was then taken out of the test tube and was separated from the RBC's layer by cutting with the help of scissors. PRF was then obtained in the form of the membrane by squeezing it in between two sterilized gauze piece.

Surgical management

The facial skin all around oral cavity was scrubbed with povidone-iodine solution and 0.2% chlorhexidine digluconate was used for intraoral antisepsis. Following local anesthesia, full thickness mucoperiosteal flap was reflected on the buccal side extending at least one tooth mesial and distal to the tooth with furcation defect. After meticulous debridement of the furcation defect and root planning, direct measurements of the osseous defects were taken using a UNC-15 periodontal probe and Naber, s probe as described [Figures 1-4].

In Group I, β-TCP bone graft (Septodont RTR™) was combined with a physiological saline solution only and condensed to a level of the plane connecting the eminences of the root surfaces adjacent to the furcation defect [Figure 5]. In Group II the osseous graft was condensed in the furcation defect and prepared PRF membrane was placed in such a way that the defect was completely covered by the membrane [Figures 6 and 7].

In both the groups, following surgery the tension-free soft tissue flaps were repositioned and secured in place by

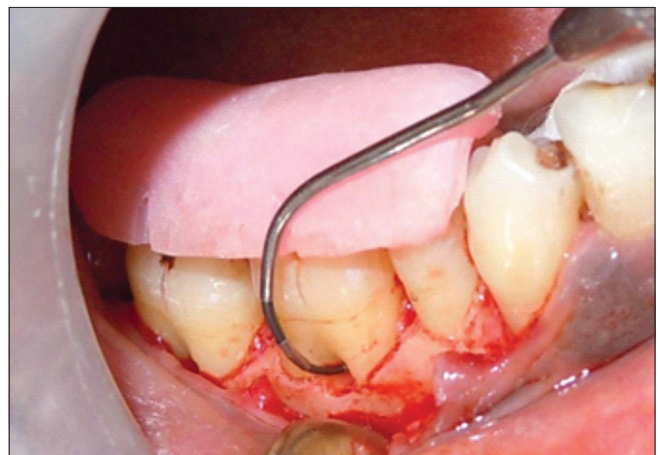Figure 1: Preoperative vertical defect depth in Group I

Figure 2: Preoperative horizontal defect depth in Group I

Figure 3: Preoperative vertical defect depth in Group II

interrupted suturing using 3–0 black silk suture material [Figures 8 and 9]. The periodontal dressing was applied to protect the surgical site.

Postsurgically, patients were prescribed antibiotics (Novamox LB 250 mg 8 hourly) and analgesics

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Figure 4: Preoperative horizontal defect depth in Group II

Figure 5: Bone graft in furcation defect Group I

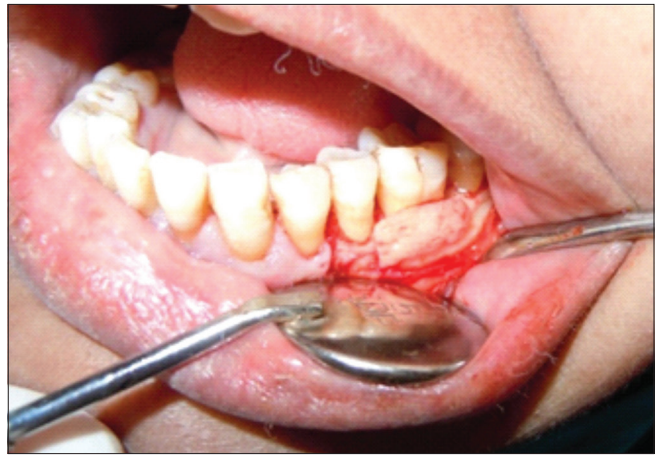Figure 6: Bone graft placed in furcation defect Group II

Figure 7: Platelet-rich fibrin membrane placed over defect Group II

Figure 8: Operated site sutured Group I

Figure 9: Operated site sutured Group II

(Ibuprofen 400 mg TID) for 5 days. Patients were instructed to rinse with 0.2% chlorhexidine gluconate (Twice daily for 4 weeks). After surgery, the patients were asked to refrain from tooth brushing, flossing, and interdental cleaning in the treated area for 4 weeks after surgery. After 10–12 days, periodontal dressing and sutures were removed. Recall appointments were then made at 15 days, 1 month and 3 months for additional follow-up and plaque control.

Reentry

Reentry surgeries were scheduled 6 months postoperatively, and all soft and hard tissue measurements were repeated with

previously used acrylic stents. According to the defects site, full thickness flap was reflected, and hard tissue measurements were recorded [Figures 10-13]. Then, the flap was repositioned and sutured with 3–0 black silk suture. The periodontal dressing was placed. Dressings and suture were removed after 1 week.

Statistical tools employed

The results were averaged (mean standard deviation [SD]) for each clinical parameter at both time intervals. The difference between each pair of measurement was then calculated (Baseline– 6 months). As the sample size was <30 for both the groups, a nonparametric evaluation plan was adopted. Comparison between both groups was performed using the nonparametric Mann–Whitney test. The statistical analysis was performed using SPSS (Statistical Package for Social Sciences) Version 15.0 Statistical Analysis Software, IBM Company, India. The values were represented in number (%) and mean ± SD.

RESULTS

Wound healing was normal with neither infectious episodes nor unpleasant clinical symptoms for both groups. Soft tissues healed within the normal limits without any complications, and no significant visual differences were noted between the treatment groups. At 6 months postoperatively, Group II presented a greater reduction in VDD and HDD and gain in CAL than Group I and GR were also less in Group II as compared to Group I. However, the reduction in PPD was more in Group I than Group II. In both the groups, mean reduction for all parameters after treatment was statistically significant; however, the difference in mean % reduction between two groups was not statistically significant.

The values of the clinical soft tissue parameters at baseline and at 6 months are shown in Table 1. At 6 months, Group I and Group II presented a mean CAL gain of 2.80 ± 1.40 mm ($P < 0.007$) and 3.00 ± 1.49 mm ($P < 0.005$), PPD reduction of 2.80 ± 1.93 mm ($P < 0.005$) and 3.50 ± 2.27 mm ($P < 0.007$), and GR of 0.70 ± 0.67 mm ($P < 0.005$) and 0.40 ± 0.52 mm ($P < 0.005$), respectively, which is statistically significant from their baseline, but the inter-group comparison revealed a statistically

Figure 10: Postoperative vertical defect depth in Group I
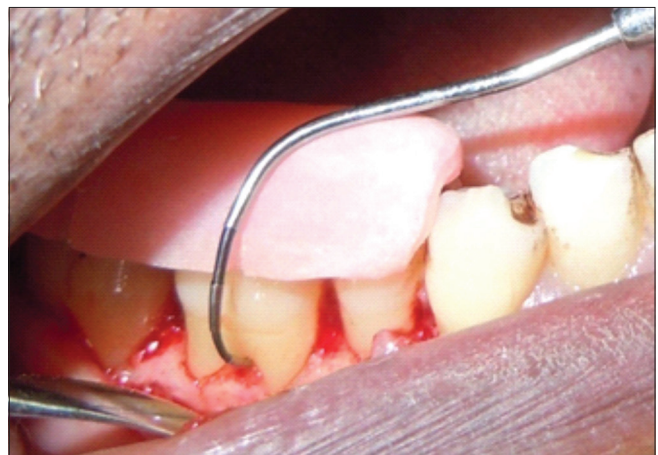

Figure 11: Postoperative horizontal defect depth in Group I

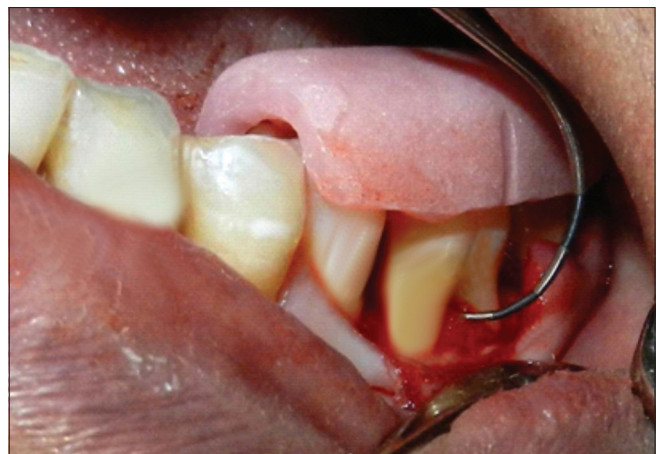Figure 12: Postoperative vertical defect depth in Group II

Figure 13: Postoperative horizontal defect depth in Group II

insignificant (*P* > 0.05) difference for all soft tissue parameters.

Evaluation of the hard tissue findings [Table 2] indicated that the treatment modality in both groups resulted in statistically significant bone fill at 6 months. Group I presented with a reduction in VDD of 3.50 ± 2.12 mm (63.85 ± 17.95%) and a HDD of 3.70 ± 0.67 mm (70.00 ± 11.97%), which is less than finding of Group II, presenting a VDD fill of 3.70 ± 1.57 mm (64.51 ± 18.72%) and a horizontal defect fill of 4.00 ± 0.88 mm (70.48 ± 12.91%) over a period of 6 months. However, the intergroup comparisons revealed a statistically insignificant difference for both hard tissue parameters.

DISCUSSION

Furcation involvement is one of the most common dentoalveolar sequelae of periodontitis, and its management presents an exclusive clinical problem in periodontal therapy. Numerous studies have confirmed that furcation involved molars respond less favorably to periodontal therapy than molars without furcation involvement or single-rooted teeth, and are at a higher rate of periodontal breakdown than other teeth.[18]

The results of the present study showed that treatment of furcation defects with both PRF+β-TCP and β-TCP alone leads to significant PPD, CAL, GR, HDD, and VDD improvement as compared to baseline values. Although no statistically significant differences in any of the investigated parameters were found between the treatments.

To the best of our knowledge, there are no studies in the literature comparing PRF membrane+β-Tcp and β-Tcp applications in the treatment of Grade II furcation defects, and therefore, direct comparison of the results is not possible. Glickman's class II furcation defects respond favorably to

Table 1: Soft tissue measurement

Parameter	Group	Mean±SD				Significance of change (Wilcoxon signed rank test)	
		Before treatment	After 6 months	Reduction	Percentage change	Z	P
CAL	Group I	6.40±2.80	3.60±2.07	2.80±1.40	42.51±20.18	2.687	0.007*
	Group II	7.00±2.98	4.00±2.40	3.00±1.49	44.05±12.88	2.829	0.005*
	Significance of difference (Mann-Whitney U-test) (Z, P)	0.434, 0.684	0.468, 0.684		0.152, 0.912		
PPD	Group I	5.60±2.59	2.10±0.57	3.50±2.27	58.60±13.75	2.818	0.005*
	Group II	6.10±2.92	3.30±1.42	2.80±1.93	42.50±19.32	2.673	0.007*
	Significance of difference (Mann-Whitney U-test) (Z, P)	0.427, 0.684	2.162, 0.052		1.946, 0.052		
GR	Group I	0.30±0.67	1.00±0.94	0.70±0.67	25.00±35.36	2.840	0.005*
	Group II	0.00±0.00	0.40±0.52	0.40±0.52	0.0†	2.814	0.005*
	Significance of difference (Mann-Whitney U-test) (Z, P)	1.451, 0.481	1.587, 0.165	-	-	-	

Level of significance: "*P*" is level of significance. *P*>0.05 (not significant), *P*<0.05 (significant). Intergroup differences significant (Mann-Whitney U-test): No superscript mark denotes any significant intergroup difference. *Significant intragroup differences (Wilcoxon-signed rank test); superscript mark denotes significant intragroup difference. †Percentage change could not be evaluated as in group II because pre-treatment values were 0. CAL: Clinical attachment level, PPD: Probing pocket depth, GR: Gingival recession, SD: Standard deviation

Table 2: Hard tissue measurement

Parameter	Group	Mean±SD				Significance of change (Wilcoxon signed rank test)	
		Before treatment	After 6 months	Reduction	Percentage change	Z	P
VDD	Group I	5.80±3.26	2.30±2.36	3.50±2.12	63.85±17.95	2.831	0.005*
	Group II	6.30±3.47	2.60±2.27	3.70±1.57	64.51±18.72	2.825	0.005*
	Significance of difference (Mann-Whitney U-test) (Z, P)	0.423, 0.684	0.283, 0.796		0.115, 0.912		
HDD	Group I	5.30±0.48	1.60±0.70	3.70±0.67	70.00±11.97	2.859	0.004*
	Group II	5.80±0.63	1.70±0.67	4.00±0.88	70.48±12.91	2.848	0.004*
	Significance of difference (Mann-Whitney U-test) (Z, P)	1.834, 0.105	0.755, 0.529		0.116, 0.912		

Level of significance: "*P*" is level of significance. *P*>0.05 (not significant), *P*<0.05 (significant). Intergroup differences significant (Mann-Whitney U-test): No superscript mark denotes any significant intergroup difference. *Significant intragroup differences (Wilcoxon-signed rank test); superscript mark denotes significant intragroup difference. SD: Standard deviation, VDD: Vertical defect depth, HDD: Horizontal defect depth

regenerative procedures, and hence, only class II furcation defect cases were selected for this study.[19]

Growth factors play an important role in cell regulation and present new options and provide additional treatment methods for regenerating lost periodontal support. For periodontal regeneration to occur, various biological events, including cell migration, adherence, multiplication, and differentiation need to occur in a well-orchestrated sequence. Polypeptide growth factors (PGFs) are biological mediators that can regulate cell multiplication, migration, and differentiation. Of all known PGFs, platelet-derived growth factors (PDGF), present in platelet concentrate were shown to exert a favorable effect on periodontal regeneration.

Autologous PRF is a recent and promising innovation in regenerative periodontal therapy. PRF is an organized dense fibrin scaffold with a specific slow release of growth factors such as TGF-β1, PDGF-AB, and VEGF and glycoproteins such as thrombospondin.[10-12] In addition, PRF was shown to act as a suitable scaffold for breeding human periosteal cells *in vitro*, which may be suitable for applications of bone tissue engineering.[20] PRF also induces the proliferation of various cells *in vitro* with the strongest induction effect on osteoblasts.[21] PFR in combination with various bone graft promoting wound healing, bone growth maturation, graft stabilization, hemostasis, and reduces healing time.[22,23] Hence, the present study was designed to evaluate the additive regenerative effect of PRF membrane with β-TCP in Grade II furcation defects.

The mean gain in CAL for individual groups showed statistically significant results over 6 months of the study. Gain in CAL was 2.80 ± 1.40 mm in the Group I and in Group II 3.00 ± 1.49 (P = 0.007), which is statistically significant post surgically. Sharma and Pradeep,[17,24] stated CAL gain of (3.31 ± 1.76) and (2.33 ± 0.48 mm) in an intrabony defect and furcation defect treated by PRF alone. Saini *et al*.,[25] in her study on intrabony defect using PRP with β-TCP reported 2.35 ± 0.32 gain in the test group and 1.70 ± 0.24 in control group, which on comparison was lower than what was observed for Group II. The results in Group I were comparable to the observations by Humagain *et al*.,[26] who reported 2.90 mm gain in CAL following treatment with perioglass alone in furcation defect. However, there was no statistically significant (P = 0.68) difference between the two groups. The possible reason for enhancing CAL in our study could be the growth factor in PRF which stimulates healing.

The PPD reduced 3.50 ± 2.27 mm (P = 0.005) and 2.80 ± 1.93 mm (P =0.008) in Group I and Group II,

respectively, which were statistically significant from the baseline but the intra-group comparison shows no statistically significance difference. Saini *et al*.,[25] reported 3.30 ± 0.29 mm reduction in PPD in the test group following treatment with PRP+β β-TCP in intrabony defect which was higher to the result of Group II of our study. As it is well documented that proper firm adaptation of the flap is necessary to create a fibrous union to the root surface, placement of PRF membrane beneath the flap made this initial adaptation difficult. This might have resulted in inferior results in the test group.

GR was found in both groups at 6 months, which was higher in Group I (0.70 ± 0.67 mm) than Group II (0.0 ± 040 mm) in Group II. However, the difference in mean % increment between two groups could not be compared in Group II pretreatment values were nil and hence percentage change could not be calculated. Pradeep *et al*.[16] demonstrated GR in his study 0.20 ± 0.63 mm in the test group and 0.30 ± 0.67 mm in the control group. Anilkumar *et al*.[27] achieved complete recession coverage with excellent tissue contour and color in 6 months. Lundquist *et al*.[28] stated that PRF provided protection against proteolytic degradation and sustained release of endogenous fibrogenic factor important for wound healing. As concluded by Del Corso *et al*.[29] placement of the PRF membrane, maintains the gingival flap in a high and stable position enhances neoangiogenesis, reduces the necrosis, and shrinkage of the flap.

The mean bone fill for both the groups also showed an increase which was statistically highly significant over the entire study period and the comparative evaluation between groups, the mean value of Group II (3.70 ± 1.57 mm) was higher than that of Group I (3.50 ± 2.1 mm), but the difference was found to be statistically insignificant (P = 0.912) between the groups. At reentry percent, defect fill was 63.85 and 64.51 in Group I and II, respectively. Lekovic *et al*.[30] used PRF with bovine graft in intrabony defect and found greater defect fill in the PRF-BPBM group (4.06 ± 0.87 mm on buccal and 3.94 ± 0.73 mm on lingual sites). Nailing *et al*.,[25] obtained 2.40 ± 0.21 mm VDD reduction in the test group and 2.05 ± 0.20 mm in control group in intrabony defects treated with PRP with β-TCP and PRP alone, respectively. Sharma and Pradeep.[17] evaluated PRF alone in intrabony defects and reported 2.66 ± 0.59 mm reduction in VDD, and also found 48.26 ± 5.72% bone fill in site treated with PRF alone.

Reentry after 6 months, the mean reduction in HDD was observed to be 3.70 ± 0.67 mm in Group I as compared to 4.00 ± 0.94 mm in Group II, which is statistically significant (P = 0.004) from baseline. However, the difference in mean % reduction between two groups

was not significant statistically ($P = 0.912$). Pradeep *et al.*[24] reported horizontal bone fill in Grade II furcation defects by 2.66 ± 0.59 mm in a PRF group compared to 1.88 ± 0.75 mm in OFD group. Dohan *et al.*[31] observed a strong differentiation in the osteoblasts and revealed a starting of mineralization process in the PRF membrane itself after 14 days. In our study, 90% (nine out of ten) furcation defects were reduced to Grade I and one of the defect remained in Grade II in both Group I and II.

CONCLUSIONS

Due to its peculiar properties, the natural fibrin biomaterial PRF has enormous potential for surgical wound healing. PRF + bone graft has been shown to be an effective regenerative material in the management of Grade II furcation, displaying a greater reduction in vertical and HDD and gain in clinical attachments. Further, studies are necessary to assess the histology of the regenerated tissue and mechanisms to maximize the growth factor delivery while using PRF.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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