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Research Article

EVALUATION OF THE EFFICACY OF AUTOGENOUS BONE GRAFT AND PLATELET-RICH FIBRIN IN THE TREATMENT OF PERIODONTAL INTRABONY DEFECTS

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A B S T R A C T

Aim: To evaluate the efficacy of autogenous bone graft and autologous Platelet- Rich Fibrin (PRF) in the management of periodontal intrabony defects.

Materials & Method: A total of 24 intraoral sites from the patients suffering from moderate to severe periodontitis with clinical and radiographic evidence of angular defects were selected. 12 defects were treated with autogenous bone graft (Group I) and 12 with autologous platelet rich fibrin (PRF) (Group II). Clinical parameters (i.e. probing pocket depth (PPD) and relative attachment level (RAL)) and radiological parameters (i.e. distance from CEJ to base of defect, CEJ to alveolar crest and crest to base of defect) were recorded at baseline, 3 months, 6 months and 9 months post operatively.

Results: A statistically significant PPD reduction and gain in RAL was recorded in both groups from baseline to 3 months, 6 months and 9 months. Defect fill was statistically significant from baseline to 6 months and 9 months in both the groups. However, defect fill was statistically significant from baseline to 3 months in group II only.

Conclusion: The autologous Platelet-Rich Fibrin (PRF) can be used effectively in the treatment of periodontal intrabony defects.

INTRODUCTION

The periodontal regeneration, that is, the formation of new bone and new cementum with supportive periodontal ligament has been somewhat an elusive goal in periodontics.[14] At present, bone grafting is the only modality of therapy for which there is histologic evidence, that bone, cementum and a functional periodontal ligament (a new attachment apparatus) can be formed on a previously diseased root surface in humans.[29] The autogenous bone graft is still considered a "gold standard" by which other grafting materials are compared, because autogenous bone includes cells participating in osteogenesis, along with it a tissue reaction is induced without inducing immunological reactions, minimal inflammatory reactions, rapid revascularization around the graft particles and potential release of growth and differentiation factors sequestrated within the grafts.[17]

Normal bone regeneration is a complex process that involves a large number of growth factors and cytokines for its regulation. To accelerate clinical translation of the same, there is an ongoing need to develop therapeutics based on regenerative technology, which can stimulate latent self-repair mechanisms in patients and harness the host's innate capacity for regeneration. Technological advancement has led to development of concentrated platelets, by means of centrifugation, popularly known as Platelet concentrates.[20]

One of the finest family of platelet concentrates appeared in France-Platelet-rich fibrin (PRF). It was developed by Choukroun J in 2001 for use in tissue healing and came to be known as the second and the latest generation of platelet concentrates.[5] Its completely autogenous nature, with no artificial biochemical agents involved, makes PRF a safe and an inexpensive treatment modality. The physiologic fibrin matrix of PRF, obtained as a result of slow polymerization, has the ability to hold various growth factors and cytokines and release them at the wound site for a prolonged time period. The leukocytes and key immune cytokines like IL- 1β, IL- 6, IL- 4 and TNF- α trapped in PRF give an anti-infectious and immune regulatory effect. All these properties make PRF a unique entity in itself. The field of periodontal therapy, though, has just begun to explore the vast benefits of PRF. Management of furcation defects, multiple gingival recessions and intrabony defects with the help of PRF have shown promising results. Various studies have proved that PRF not only stimulates osteoblasts, gingival fibroblasts and periodontal ligament (PDL) cell growth but also retards epithelial cell proliferation. [18]

Considering the soft tissue and hard tissue healing potential of PRF and osteogenic power and potential of autogenous bone

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graft, the current study was done to evaluate the efficacy of autologous Platelet-Rich fibrin (PRF) and autogenous bone graft in the treatment of intrabony defects.

MATERIALS & METHOD

A randomized clinical study was conducted in the Department of Periodontics, Guru Nanank Dev Dental College, Sunam. Ethical clearance was obtained from the institutional ethical committee. After ethical approval, all the patients received verbal information regarding participation and a written informed consent was then obtained. A total of 24 intraoral sites from the patients in the age group of 20-50 years, suffering from moderate to severe periodontitis with clinical and radiographic evidence of angular defects were recruited in the study.

Patients presenting with 2 and/or 3 walled intrabony periodontal defects, who had not undergone any type of regenerative periodontal therapy 6 months prior to study, with tooth mobility not more than Miller's Grade I, non- lactating, non- smokers, no known allergy to any of the test material, systemically healthy with no history of taking antibiotics 3 months prior to study were included.

Pre-Surgical Procedures: All the selected patients, following an initial examination, treatment planning and discussion were given detailed instructions in self performed plaque control measures and were subjected to Phase I periodontal therapy. After phase I periodontal therapy, the selected sites were divided into two groups:

Group I - in which the intrabony defects (IBD) were treated with Autogenous Bone Graft (ABG),

Group II - in which the intrabony defects (IBD) were treated with Autologous Platelet Rich Fibrin (PRF)

Intrabony defect after debridement Group I

Figure 1

Four to six weeks after Phase I periodontal therapy the patients were subjected to the surgical procedure. Routine haematological investigations were carried out in all the subjects.

Clinical parameters: Probing pocket depth (PPD) and relative attachment level (RAL) were recorded at baseline and at 3 months, 6 months and 9 months after surgery.

Radiographic parameters: Radiographic defect fill was measured using intraoral periapical radiograph (IOPA) using long cone/paralleling technique. The radiographs were standardized using Rinn- XCP (extension cone paralleling) instrument and a radiographic grid calibrated in millimetres. The distance was measured by counting the millimetre markings on the radiograph. The radiographs were taken at the baseline, 3 months, 6 months and 9 months post-operatively. The following calculations were made from the radiographic measurements recorded: CEJ to Base of Defect, CEJ to alveolar crest and Radiographic defect fill (i.e. distance from CEJ to base of the defect - distance from CEJ to alveolar crest).

Surgical Procedure

All the patients were premedicated [(Inj. Diazepam 10 mg (2ml) and Inj. Atropine 0.65 mg (1ml)] i.m., one hour before surgery. A pre-surgical rinse with 0.2% chlorhexidine digluconate mouthrinse for 30 seconds was done. After administering local anesthesia (lignocaine 2% with adrenaline 1:2,00,000), crevicular incisions were given at the defect site, extending one tooth adjacent to the involved tooth, both mesially and distally using a Bard Parker blade and handle. A full thickness mucoperiosteal flap was reflected to provide access to the intrabony defect and surrounding alveolar bone. The defect was thoroughly debrided with the help of surgical curettes to obtain a smooth hard surface. The surgical site was then irrigated with normal saline and inspected for any granulation tissue or deposits. Any adherent granulation tissue was trimmed from the flap. The selected sites were randomly divided (by coin-toss method) into two experimental groups: Group I IBDs (n = 12) treated open flap debridement (OFD) and filled with Autogenous bone graft (Figure 4) and Group II IBDs (n = 12) treated OFD and filled with Autologous Platelet-Rich Fibrin Graft (PRF) (Figure 8). The mucoperiosteal flaps were repositioned and sutured followed by application of the periodontal dressing (Coe- pack).

Procurement of the Grafting Material

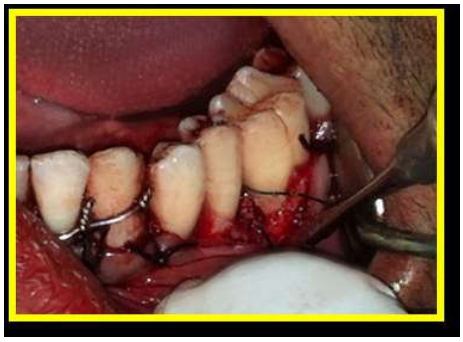
An autogenous bone graft was harvested intraorally from different sites with the help of reduction hand piece with 1/20 reduction, and a trephine bur (TRE02, Trephine Bur - 2mm INSIDE Diameter X 3mm OUTSIDE Diameter and TRE04, trephine bur 4mm INSIDE Diameter X 5mm OUTSIDE Diameter) (Figures 2 and 3).

Procurement of Autogenous Bone Graft by Trephine Bur

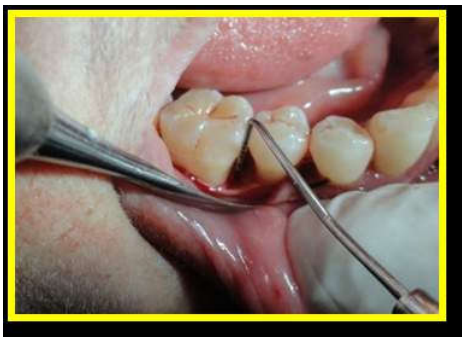
Figure 2

Autogenous Bone graft collected by trephine bur

Figure 3



Autogenous Bone Graft placed in intrabony defects (Group I)
Figure 4

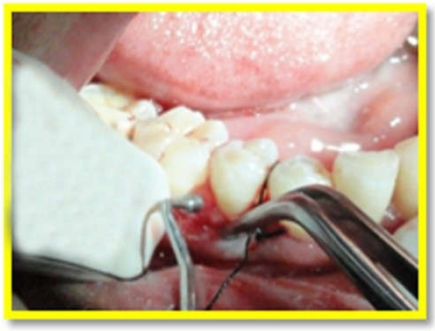


Intrabony defect after debridement Group II
Figure 5

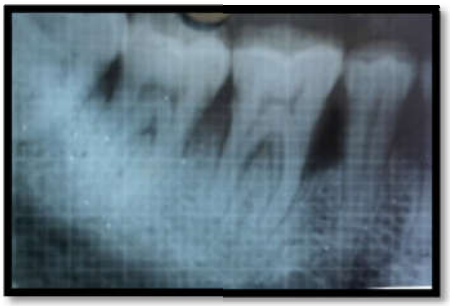
An autologous PRF graft: Platelet rich fibrin was prepared according to the protocol developed by Choukouran *et al*. (3000 RPM for 10 min) using REMI Laboratories (India) table top centrifuge (Figures 6 and 7)

Drawing of blood for preparation of platelet rich fibrin
Figure 6

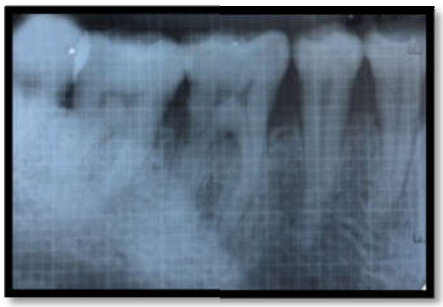
Platelet rich fibrin obtained after centrifuging the blood
Figure 7



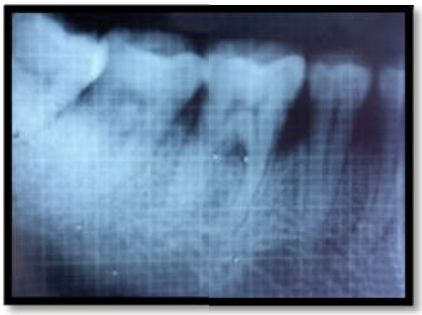
Platelet rich fibrin placed in intrabony defects (Group II)
Figure 8



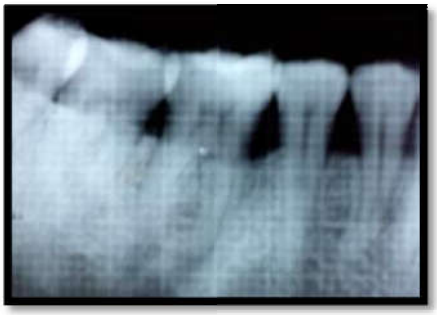
Group I At Baseline
Figure 9 (a)



Group I At 9 months
Figure 9 (b)



Group II At Baseline
Figure 10 (a)



Group II After 9 months
Figure 10(b)

OBSERVATIONS AND RESULTS

All the subjects reported uneventful healing at all the sites. The results obtained depicts changes in both soft and hard tissue parameters after 9 months for Group I and Group II, IBDs, both clinically as well as radiographically recorded at baseline, 3 months, 6 months and 9 months postoperatively. The recorded data was put to statistical analysis and the results obtained were compared.

Probing Pocket Depth (PPD)

At baseline the mean PPD for Group I (autograft) was 6.08 ± 1.24 mm which was reduced to 4.83 ± 1.26 mm, 3.50 ± 0.79 mm and 3.25 ± 0.62 mm at 3, 6 and 9 months respectively, giving statistically highly significant results in PPD reduction from baseline to 9 months. In Group II (PRF) the mean PPD at baseline was 5.83 ± 1.03 mm which reduced to 4.58 ± 0.99 mm, 3.83±0.71 and 3.58 ± 0.66 mm at 3, 6 and 9 months, respectively (Table 1). The PPD reduction was statistically highly significant ($p<0.05$) from baseline to 9 months (Table 2).

Table 1 Mean PPD and standard deviation (SD) for group I and group II at different time intervals

Groups		N	Mean	Std. Deviation	Minimum	Maximum
Autograft	Baseline	12	6.08	1.240	5.00	9.00
	3 Months	12	4.83	1.267	3.00	7.00
	6 Months	12	3.50	.798	2.00	5.00
	9 Months	12	3.25	.622	2.00	4.00
PRF	Baseline	12	5.83	1.030	4.00	7.00
	3 Months	12	4.58	.996	3.00	6.00
	6 Months	12	3.83	.718	3.00	5.00
	9 Months	12	3.58	.669	3.00	5.00

Table 2 Comparison of mean of change in PPD at different time intervals in both the groups

Variable	Groups	Mean	Std. Deviation	P value
Baseline to 3 months	Autograft	1.2500	.96531	.951
	PRF	1.2500	.75378	
Baseline to 6 months	Autograft	2.5833	1.16450	.227
	PRF	2.0000	.85280	
Baseline to 9 months	Autograft	2.8333	.93744	.189
	PRF	2.2500	.96531	
3 months to 6 months	Autograft	1.3333	.77850	.059
	PRF	.7500	.62158	
3 months to 9 months	Autograft	1.5833	.79296	.105
	PRF	1.0000	.73855	
6 months to 9 months	Autograft	.2500	.45227	1.000
	PRF	.2500	.45227	

Relative attachment level (RAL)

The mean RAL for Group I (autograft) the results showed statistically highly significant change in RAL in Group I from baseline to 9 months ($p< 0.05$). For Group II (PRF) the mean RAL at baseline was 11.92 ± 1.78 mm, which was reduced to 10.83 ± 1.64 mm, 9.42 ± 1.44 mm and 9.08 ± 1.16 mm at 3, 6 and 9 months, (table 3) showing statistically highly significant change in RAL from baseline to 9 months ($p< 0.05$) (Table 4).

Table 3 Mean and standard deviation (SD) of RAL for group I and group II at different time intervals

Groups		N	Mean	Std. Deviation	Minimum	Maximum
Autograft	Baseline	12	11.17	1.946	8	14
	3 Months	12	9.92	1.676	8	13
	6 Months	12	8.33	1.826	6	12
	9 Months	12	7.83	1.337	6	10
PRF	Baseline	12	11.92	1.782	10	15
	3 Months	12	10.83	1.642	9	14
	6 Months	12	9.42	1.443	8	12
	9 Months	12	9.08	1.165	8	11

Table 4 Comparison of means of change in RAL for both groups at different time intervals

Variable	Groups	Mean	Std. Deviation	P value
Baseline to 3 months	Autograft	1.2500	.86603	.512
	PRF	1.0833	.66856	
Baseline to 6 months	Autograft	2.8333	1.02986	.363
	PRF	2.5000	1.00000	
Baseline to 9 months	Autograft	3.3333	.77850	.187
	PRF	2.8333	1.11464	
3 months to 6 months	Autograft	1.5833	.51493	.633
	PRF	1.4167	.90034	
3 months to 9 months	Autograft	2.0833	.79296	.435
	PRF	1.7500	.96531	
6 months to 9 months	Autograft	.5000	.90453	.974
	PRF	.3333	.65134	

Bone defect fill - The mean crest to base of defect distance for Group I at baseline was 1.75 ± 0.62mm which was reduced to 1.41 ± 0.79 mm, 0.91 ± 0.79 mm and 0.41±0.51mm at 3, 6 and 9 months, respectively. The mean reduction observed from baseline to 9 months in Group I was statistically highly significant ($p<0.003$). The mean crest to base of defect distance for Group II at baseline was 1.83 ± 1.02 mm which was reduced to 1.08 ± 0.99 mm, 0.67 ± 1.37 mm and 0.67 ± 0.89 mm at 3,6 and 9 months, showing statistically significant results from baseline to 9 months ($p<0.012$). (Table 5 and 6)

Inter-group Comparison

During the study, a statistically significant PPD reduction and gain in RAL was seen in both the groups, that is, Group I and Group II, from baseline to 3 months, 6 months and 9 months. However, on comparison between the two groups, the change in PPD reduction and gain in RAL was not statistically significant.

Table 5 Mean and SD of crest to base of defect for both the groups at different time intervals

Groups		N	Mean	Std. Deviation	Minimum	Maximum
Autograft	Baseline	12	1.7500	.62158	1.00	3.00
	3 Months	12	1.4167	.79296	.00	3.00
	6 Months	12	.9167	.79296	.00	2.00
	9 Months	12	.4167	.51493	.00	1.00
PRF	Baseline	12	1.8333	1.02986	1.00	4.00
	3 Months	12	1.0833	.99620	.00	3.00
	6 Months	12	.6667	1.37069	-1.00	4.00
	9 Months	12	.6667	.88763	.00	2.00

Table 6 Comparison of means of change in crest to base of defect for both groups at different time intervals

Variable	Groups	Mean	Std. Deviation	P value
Baseline to 3 months	Autograft	.3333	.77850	.221
	PRF	.7500	.62158	
Baseline to 6 months	Autograft	.8333	.83485	.442
	PRF	1.1667	1.19342	
Baseline to 9 months	Autograft	1.3333	.65134	.515
	PRF	1.1667	1.26730	
3 months to 6 months	Autograft	.5000	.79772	.808
	PRF	.4167	1.56428	
3 months to 9 months	Autograft	1.0000	.42640	.113
	PRF	.4167	1.31137	
6 months to 9 months	Autograft	.5000	.67420	.316
	PRF	.0000	1.20605	

During the study both the groups showed significant bone fill at different time intervals. On comparing the bone fill defect between Group I and Group II, the results were not statistically significant. Table 7 shows that, though the percentage change

in bone fill was greater in Group I (percentage Mean change in bone fill = 76.38%) from baseline to 9 months as compared to Group II (percentage mean change in bone fill = 55.55%) (Table 7) but values were statistically not significant.

Table 7 % change in crest to base of defect in both the groups at different time intervals

Variable	Groups	% change
Baseline to 3 months	Autograft	15.27
	PRF	45.13
Baseline to 6 months	Autograft	44.44
	PRF	79.16
Baseline to 9 months	Autograft	76.38
	PRF	55.55
3 months to 6 months	Autograft	43.93
	PRF	37.03
3 months to 9 months	Autograft	78.78
	PRF	59.25
6 months to 9 months	Autograft	50.00
	PRF	81.25

DISCUSSION

In the present study, an attempt has been made to evaluate and compare, clinically and radiographically the regenerative potential of autogenous bone graft (cortico-cancellous) harvested intraorally with a trephine bur and autologous PRF, as a sole graft material in the treatment of intraosseous defects in moderate to severe periodontitis patients.

The 9 month study period was in accordance with the studies done by Robinson E (1969),[31] Yilmaz S *et al* (2010),[40] Paolantonio M *et al* (2010),[26] Guida L *et al* (2007),[13] Orsini M *et al* (2008),[23] Pradeep AR *et al* (2011),[27] Chitsazi MT *et al* (2011)[3] and Thorat M *et al* (2011)[37] considering tissue maturation, connective tissue healing and bone formation. A 9 month study may be needed to confirm the stability of the clinical outcome because of the high resorption rate of autogenous bone graft in the initial phases of healing.[38] The graft appears to reach its maximum radio opacity at 8 months to 1 year after treatment.[12]

Patients selected for the study presented with moderate to severe periodontitis, with good general physical health, adequate levels of oral hygiene maintenance and were non smokers. Clinical studies suggest that patients exhibiting high oral hygiene standards maintain their teeth in healthy conditions for long periods of time, perhaps even a lifetime.[1]

Healing was uneventful in all the cases. These findings are in agreement with the results of the histological and clinical studies which have shown that neither of the graft material i.e. autogenous graft and PRF elicits allergic or foreign body reaction.[41,31,30] Clinical parameters like probing pocket depth (PPD) and relative attachment level (RAL) and radiographic measurements were recorded at 3, 6 and 9 months follow up visits.

This study has found a clinically successful use of autogenous bone graft (Group I) harvested from intraoral sites in the treatment of intrabony defects similar to various investigators like Nabers CL and O'Leary TJ (1965),[22] Ellegard B *et al* (1971),[8] Rosenberg MM (1971),[33] Hiatt WH (1973),[15] Froum SJ *et al* (1975),[9] Schallhorn RG (1977)[34] and Stahl SS *et al* (1983)[36] who have successfully used the graft.[32]

The PRF (Group II) used in this study has shown successful results similar to the other researchers like Lekovic V *et al* (2011),[19] Pradeep AR *et al* (2011),[27] Sharma A *et al*[35] and Thorat M *et al* (2011).[37]

There was statistically highly significant reduction in PPD in both the groups over different time intervals. In the treatment of periodontitis, reduction in probing depth occurs through either gingival margin recession or relative gain in clinical attachment. There is reduction in the inflammation of the gingival tissues after the therapy. This is in accordnce with the studies done by Froum SJ *et al* (1975, 1976)[10,11], Trombelli L *et al* (2006),[38] Guida L *et al* (2007) [29] and Pagliaro U *et al* (2008).[24] On intergroup comparison the improvement shown by Group I in comparison to Group II was not statistically significant at 3, 6 and 9 months of the study.

This study showed a statistically highly significant ($p < 0.05$) gain in RAL in both the groups. In the treatment of periodontitis, the gain in relative attachment level seems to be achieved by epithelial and connective tissue adhesion- coronal to the bottom of treated intrabony defect.[42]

When the change in RAL was compared between two groups; the improvement shown by Group I in comparison to Group II was not statistically significant.

A defect fill of 1.33mm (76.38%) was found in Group I at 9 month evaluation. Defect fill has been seen after using autograft in studies done by Robinson E (1969),[31] Rosenberg MM (1971),[33] Hiatt WH and Schallhorn RG (1973),[15] Ellegard B and Loe H (1971)[8] and Guida L *et al* (2007).[13] The grafted autogenous bone used in the study improved the healing outcomes regarding probing depth reduction, gain in relative attachment and osseous defect fill. This can be attributed to the bone blend form and the cortico-cancellous nature of the graft, obtained by trephine bur (which was later reduced to particulate state), providing structural support, all elements of regeneration, minimal complications and low failure rate. Thus, justifying the fact that, autogenous bone graft material has a high osteogenic potential and no immunological reaction.[25]

The bone fill of 1.16mm (55.55%) was seen from baseline to 9 months in Group II. Similar results were shown by Tharot M *et al* (2011),[37] Chang YC *et al* (2011)[2] and Lekovic V *et al* (2012),[19] Pradeep AR *et al* (2011)[27] and Sharma A *et al* (2011).[35] In the present study, PRF application exhibited the radiographic intensity increase by periapical radiography after 6 months over lesion areas. This shows that PRF results in significant periodontal osseous defect healing, which may be explained as follows: PRF has been found to upregulate phosphorylated extracellular signal-regulated protein kinase expression and suppress osteoclastogenesis by promoting the secretion of osteoprotegerin in osteoblasts cultures.[2] PRF was also demonstrated to stimulate osteogenic differentiation of human dental pulp cells by upregulating osteoprotegerin and alkaline phosphatase expression.[16] Furthermore, many growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor (TGF-β) are released from PRF.[6,21,39] Mazor Z *et al*, 2009 and Tsai J *et al*, 2009 have demonstrated that PRF has a slow and sustained release of key growth factors for at least 7 days and up to 28 days which means that the PRF stimulates its environment for a significant time during remodeling.[21,39] The properties of this natural fibrin biomaterial thus offer great potential during wound healing. Choukroun J *et al* in 2006 have demonstrated that fibrin matrix leads directly to angiogenesis.[4] According to

Dohan DM *et al*, 2006 fibrin constitutes a natural support to immunity and reduces inflammatory process.[18] PRF itself can be recognized as an autologous biomaterial. As a grafting material, PRF offers an improved space making effect of the barrier, which is conducive to cell events leading to periodontal regeneration and facilitation of mineralized tissue formation due to osteoconductive and/or osteoinductive properties, possibly inherent in PRF.[7]

A significant bone fill was observed in both the groups. Though, the bone fill was greater in Group I but on comparison between the groups, the results were statistically not significant. As, the amount of crestal bone resorption was minimal, these changes mostly reflect the filling of the intrabony defects.

PRF is an autogenous preparation, obtained from patient's own blood, thus, an unlimited source of graft material could be expected, eliminating the need for the second (donor) surgical site and causing less post surgical discomfort, making it a safer treatment modality for the patient. PRF grafting is less technique sensitive procedure than GTR or bone graft placement regenerative therapy.[28] Therefore, our study may suggest that the use of PRF can be considered as a simple, inexpensive and a minimally invasive technique that seems to be a clinically relevant and very promising option as a grafting material in periodontal osseous defects.

CONCLUSION

Thus, within the limitations of present study, both treatment modalities, autogenous bone graft and Platelet-Rich Fibrin (PRF), used as a sole filling material showed a definite improvement in all clinical parameters and radiographic parameters. However, assessment of periodontal regeneration requires histological evidence, which could not be carried out in the present study because of the ethical considerations and patient management limitations.

Therefore, further studies with higher number of subjects, long term observations and histological evaluation should be one

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