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Clinico - Radiographic Evaluation of Two Forms of PRF along with DFDBA Bone Graft in the Treatment of Periodontal Intrabony Defects

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Abstract: ***Background**: Man's insatiable desire to create the lost part has led to a plethora of opportunities in periodontal regenerative treatment. One such name, platelet - rich fibrin (PRF) is an autologous concentration of human platelets which along with the platelet derived growth factor (PDGF) has been found to be more favourable for periodontal regeneration amongst all the growth factors found in plasma. **Objective**: The present study was aimed at comparing the clinical and radiographic evaluation of Leukocyte PRF (3000 rpm; 12 minutes) and Advanced PRF (1500 rpm; 14 minutes) along with demineralized freeze dried bone allograft (DFDBA) in the treatment of periodontal intrabony defects. **Materials and Methods**: Forty chronic periodontitis patients with intrabony defects (IBDs) were randomly treated by L - PRF or A - PRF with DFDBA. Probing pocket depth (PPD), clinical attachment level (CAL) and radiographic bone fill (RBF) were recorded at baseline, three and six months post - surgery. **Results**: The mean PPD reduction was greater in the A - PRF group (2.43±0.69 mm) than in the L - PRF group (1.65±0.78 mm) and the mean CAL gain were 2.43±0.67, 1.78±0.91 mm respectively. Greater percentage of mean bone fill was found in the A - PRF group (35.27%) compared to the other group (20.71%). **Conclusion**: Advanced PRF can be used predictably to reconstruct the lost periodontal structures as indicated by PPD reduction, CAL gain, intrabony defect fill and gives more definitive outcome than L - PRF.*

Keywords: Allograft; Chronic periodontitis; Centrifugation; Growth factors; Osteoinduction

1. Introduction

Periodontitis is an inflammatory disease of the periodontium and is marked by the irreversible loss of connective tissue attachment and supporting alveolar bone. [1] The final aim of periodontal therapy is to regenerate the lost periodontal tissues and thus restore function. Periodontal regeneration can be defined as the complete restoration of lost tissues to their original architecture and function by recapping the important wound - healing events associated with their development. [2] The most common form of regenerative periodontal therapy is the use of bone grafts. Bone grafting materials function as structural scaffolds and matrices for attachment and proliferation of anchorage - dependent osteoblasts. [3] Regardless of the success demonstrated with autogenous bone graft, the use of such grafts is frequently either impractical or impossible due to the difficulty to obtain sufficient autogenous bone. It is then that the allografts come to the rescue. One of the commonly used allografts is demineralized freeze - dried bone allograft (DFDBA). It stimulates bone formation by the processes of osteoinduction and osteoconduction. [3]

Platelet - rich fibrin (PRF) is an autologous concentration of human platelets and has been found to be more favorable for periodontal regeneration. PRF is a slowly and naturally polymerizing fibrin matrix in which growth factors like platelet - derived growth factor (PDGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF) and insulin - like growth factor (IGF), leukocytes, cytokines (interleukins [IL - 1, 6, 4]) and tumour necrosis factor (TNF - α) are present. [4] Ghanaati et al conducted an in - vitro study comparing standard platelet rich fibrin (S - PRF) (2700 rpm, 12 minutes) and advanced platelet - rich fibrin (A - PRF) (1500 rpm, 14 minutes) and concluded that decreasing the rpm while increasing the centrifugation time in the A - PRF group resulted in an enhanced presence of neutrophilic granulocytes and platelets in the distal part of the clot which might influence bone and soft tissue regeneration, especially through the presence of monocytes/ macrophages and their growth factors. [5]

Hence the present study was conducted to compare the clinical and radiographic evaluation of leucocyte PRF (L - PRF) (3000 rpm; 12 minutes) and A - PRF protocol (1500 rpm; 14 minutes) along with DFDBA bone graft material in the treatment of periodontal intrabony defects.

2. Materials and Methods

A total of forty intrabony defects in forty patients with chronic periodontitis as diagnosed by clinical examination and radiographs were selected for the study. A detailed case history was recorded in a specially prepared proforma which included information regarding the patient's overall medical status/general health, oral status and well - being. The nature and purpose of the study was explained to the patients in their native language and a written informed consent was taken. The study was approved by research and institutional ethical committee. The inclusion criteria were patients with chronic periodontitis within 30 - 55 years of age, minimum 20 permanent teeth should be present, periodontal pocket depth ≥ 5mm, evidence of angular defects as determined by IOPA. The exclusion criteria were systemically compromised patients and those on medications that may interfere with wound healing, pregnant women and lactating mother, active periodontal treatment within last six months and smokers.

Initially, a full mouth scaling and root planing procedure was performed for all patients and each patient was given careful

instructions regarding oral hygiene measures. They were reviewed after 4–6 weeks of Phase I therapy for a detailed periodontal evaluation. A single operator performed all surgeries as well as clinical and radiographic measurements. Forty subjects with one intrabony defect each were chosen for the study. The subjects were randomly assigned by coin test into two groups. Twenty intrabony defects were treated by placement of L - PRF (3000 rpm; 12 minutes) with DFDBA bone graft in one group while the rest 20 intrabony defects were treated by placement of A - PRF (1500 rpm; 14 minutes) with DFDBA bone graft.

Gingival index (GI), probing pocket depth (PPD), clinical attachment level (CAL) and intrabony defect fill (IBD) were recorded at baseline and again after three and six months of the surgery. [6] Prior to the surgery, study casts were prepared and the customized acrylic stent was fabricated for each subject and stored appropriately to minimize distortion. The stent was grooved so that the position and angulation of manual UNC - 15 periodontal probe can be replicated for each successive measurement. The UNC - 15 periodontal probe was used to measure the PPD from the gingival margin to the base of the periodontal pocket and the CAL was measured from the cemento enamel junction (CEJ) to the base of the periodontal pocket [Fig 1]. The radiographs were taken along with grid and standardized by using long cone paralleling technique and using film holders (RINN XCPTM, DENTSPLY) [Fig 2]. Vista scan machine was used to develop the digital radiographs. IBD was measured on the radiograph by measuring the vertical distance from CEJ of the tooth to the base of the defect by using special software [University of xx (xx) Image Tool]. The IBD depth was evaluated at baseline, three and six months.

PRF preparation

After taking informed written consent of the patient, around 5 ml of whole venous blood is withdrawn from antecubital vein transferred into 5ml sterile glass test tube without adding anticoagulant and placed in a centrifugal machine. L - PRF was prepared at 3000 rpm for 12 minutes and A - PRF was prepared at 1500 rpm for 14 minutes. Because of differential densities, it resulted in the separation of three basic fractions: a base of red blood cells at the bottom, acellular plasma on the surface and finally a PRF clot between the two. A total of 2–3ml of the top layer was pipette out with the sterile dropper; the middle layer (PRF) was removed with a tweezer and placed in a sterile dappen dish and used along with the DFDBA bone graft.

After anesthetizing the area with 2% lignocaine with adrenaline (1: 80000) solution, a sulcular incision is given and a full thickness mucoperiosteal flap was elevated [Fig 3]. Then thorough debridement was performed using area - specific curettes and the anatomy of the intrabony defect was clinically confirmed and the defect was filled either with L - PRF with DFDBA or A - PRF with DFDBA according to the group assigned to the defect [Fig 4]. The buccal and lingual flaps were approximated using a 3 - 0 non - resorbable sutures and periodontal dressing was given. All patients received systemic antibiotic therapy (loading dose of Doxycycline 200 mg followed by 100 mg once daily for 5 days) and analgesics (Ketorolac twice daily) for three days to prevent post - operative pain and oedema. Post - operative instructions were given to the patient. Local plaque control was maintained by 0.2% chlorhexidine rinse twice daily. Pack and sutures were checked and removed seven days after the surgery. The area was irrigated thoroughly with 0.9% normal saline. Healing of flap was visualized and symptoms regarding discomfort, pain and swelling were asked to the patient. No attempt to probe was made before the three months follow up examination. The PPD, CAL and IBD depth were evaluated at three and six months [Figs 5, 6].

Statistical analysis

The intragroup comparison of clinical parameters like gingival index, probing pocket depth, clinical attachment level, and radiographic defect fill were compared by using dependent t - test & intergroup comparison was done by using independent t - test [Tables 1 & 2]. Differences were considered as statistically significant at $p<0.05$*.

3. Results

All participants completed the study with no post - operative complications reported during the study period. Both L - PRF and A - PRF produced a statistically significant reduction in PPD, gain in CAL and radiographic bone fill from baseline to six months (Table 2). The change in GI was statistically not significant.

4. Discussion

Platelet - rich fibrin (PRF) has been introduced by Choukroun *et al.* [7] It is a second - generation autologous platelet concentrate. It has several advantages over PRP. It is produced in a totally natural manner, without using anticoagulant during blood harvest for platelet activation and fibrin polymerization. The absence of anticoagulant implies the activation of most platelets of the blood sample in contact with the tube walls and release of the coagulation cascades within a few minutes. The protocol is very simple and of low cost.

Biological rationale of PRF: The scientific rationale behind the use of platelet preparations lies in the fact that the platelet alpha granules are a reservoir of many growth factors that are known to play a crucial role in hard and soft tissue repair mechanism. [6] These include platelet - derived growth factors (PDGFs), transforming growth factor beta (TGF-□), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin - like growth factor-1 (IGF-1) etc. Platelet growth factors exhibit chemotactic and mitogenic properties that promote and modulate cellular functions involved in tissue healing and regeneration and cell proliferation. These products of the healing cascade are considered to be important for optimum healing. [8]

L - PRF is produced at a speed of 3000 rpm for 12 minutes in sterile glass based plastic tubes. For formation of A - PRF, slower speed (1500rpm) and more time (14 min) was used in sterile plain glass - based vacuum tubes. David et al (2014) have proposed that this new protocol (A - PRF) leads to enhanced B and T lymphocytes entrapment, more even distribution of platelets and neutrophils. [9] They also stated that the number of viable cells including platelets are much higher in A - PRF. There is better development of resident

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monocytes, macrophages and lymphocytes. Clinically, this would be beneficial as it would translate into an increased amount of growth factor and cytokine release. However, some studies have provided contradictory results. Pinto *et al* demonstrated that A - PRF protocol produced lighter, shorter, narrower clot with light polymerization and more squashed bodies. [10] When the growth factors (TGF, PDGF, VEGF) released from A - PRF were compared to that of L - PRF it was found that the levels were less than half of those from L - PRF. However, in another study Kobayashi *et al* stated that A–PRF released significantly higher total quantities of growth factors when compared to traditional PRF. [11] There is limited literature on the comparison between the two protocols and more studies are required to ascertain the benefits and limitations of L - PRF vs A - PRF.

Sakshi *et al* concluded from their study that a combination of PRF and DFDBA demonstrated significant improvement in the clinical probing depth, relative attachment level and radiographical bone fill and which is in accordance with the present study. [12] In a recent systematic review and meta - analysis on the regenerative potential of L - PRF in intra - bony defects, Castro *et al* reported that significant PPD depth reduction (1.1±0.5 mm, p< 0.001), clinical attachment gain (1.2±0.6 mm, p<0.001) and bone fill (1.7±0.7 mm, p<0.001) were found when comparing L - PRF to open flap debridement (OFD) in intrabony defects. [13]

In the present study, the reduction of mean PPD in the L - PRF group was from 5.90±0.81 at baseline to 4.25 ± 0.65 at 6th month, whereas in the other group was from 5.97 ± 0.78 to 3.53 ±0.30 respectively. The mean CAL in the L - PRF group was 6.42±0.99 at baseline, which reduced to 4.63 ± 0.75 at 6th month whereas in the other group was from 6.18 ± 0.82 at baseline to 3.75 ±0.34 at 6th month. The mean radiographic defect depth in L - PRF group was 7.94±2.50 at baseline, which reduced to 6.30 ± 1.78 at 6th month, whereas A - PRF group has shown the radiographic defect depth reduction from 9.30 ± 1.94 at baseline to 6.02 ±1.02 at 6th month interval. In the present study, both the groups showed significant improvement in clinical and radiographic parameters. However, the A - PRF group showed significant enhancement of both clinical and radiographic outcomes.

These results may be attributed to beneficial effects of new platelet rich fibrin protocol (A - PRF) introduced by Choukroun *et al* where they conducted an *in - vitro* study to assess histologically and histo - morphometrically the cell distribution pattern between standard PRF protocol (2700 rpm, 12 minutes) and the advanced PRF protocol (1500 rpm, 14 minutes) and they concluded that in the longitudinal section of the S - PRF clot, a dense fibrin clot was seen with minimal interfibrous space. With the standard histochemical staining methods, cells were observed throughout the clot and decreasing toward the more distal parts of the PRF clot. PRF clots formed with the A - PRF centrifugation protocol showed a looser structure with more interfibrous space and more cells could be counted in the fibrin - rich clot. Furthermore, the cells were more evenly distributed throughout the clot as compared to S - PRF.

Masako Fujioka *et al* investigate the influence of centrifugation speed (g - force) and time on PRF matrix scaffolds, their release of growth factors as well as their effect on cellular biocompatibility and activity. [14] In their study, Standard L - PRF served as a control (2700rpm - 12 minutes). Two test groups utilizing low - speed (1300rpm - 14 min termed advanced - PRF, A - PRF) and low - speed+ time (1300rpm - 8 min; A - PRF+) were investigated. Each PRF matrix was tested for growth factor release up to ten days as well as biocompatibility and cellular activity. The low - speed concept (A - PRF, A - PRF+) demonstrated a significant increase in growth factor release of PDGF, TGF - β1, EGF and IGF with A - PRF+ being highest of all groups. While all PRF formulations were extremely biocompatible due to their autogenous sources, both A - PRF and A - PRF+ demonstrated significantly higher levels of human fibroblast migration and proliferation when compared to L - PRF. They concluded that modifications to centrifugation speed and time with the low speed concept was shown to favour an increase in growth factor release from PRF clots which in turn may directly influence tissue regeneration by increasing fibroblast migration, proliferation and collagen mRNA levels. Another study by Kobayashi *et al* evaluated the comparative release of growth factors from PRP, PRF and advanced - PRF and reported that the new formulation of PRF (A - PRF) released significantly higher total quantities of growth factors when compared to traditional PRF. [9]

Thus, in the present study Advanced PRF with DFDBA demonstrated better results in PPD reduction and CAL reduction and radiographic defect depth reduction as compared to L - PRF with DFDBA. This result may be attributed to beneficial effects of A - PRF protocol which has modifications in centrifugation time and speed which would have had potentially beneficial effects on regeneration in intrabony defects compared to L - PRF protocol as mentioned in above studies.

5. Conclusion

Studies comparing L - PRF to A - PRF are sparse. The limitations of the present study include small sample size and lack of more advanced radiographic techniques like CBCT which would have given more accurate calibrations. Quantification and release of growth factors from PRF and histomorphometric analysis were not carried out. Within the limitations of this study, it can be concluded that A - PRF protocol enhances regeneration in the treatment of intrabony defects compared with L - PRF protocol.

Conflict of interest - NIL

Financial disclaimer - Self - funded study

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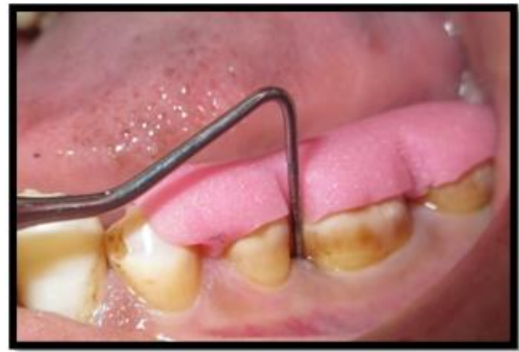

Figure 1: Measurement of probing pocket depth at base line

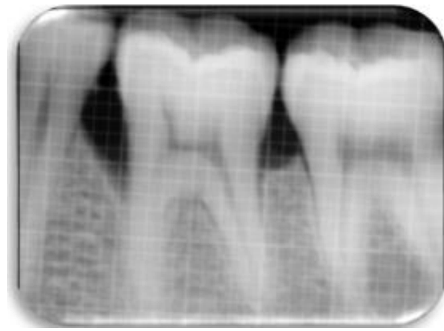

Figure 2: Measurement of intrabony defect fill at base line

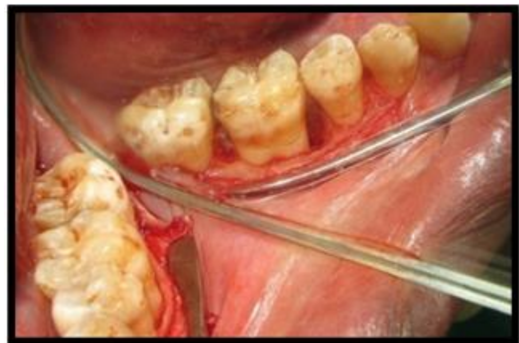

Figure 3: Flap elevation and debridement

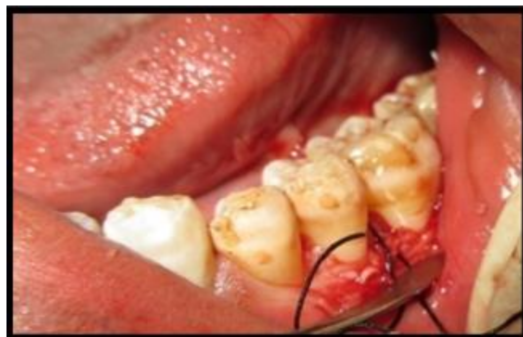

Figure 4: PRF with DFDBA graft placement into the defect

Figure 5: Measurement of probing pocket depth at 6 months

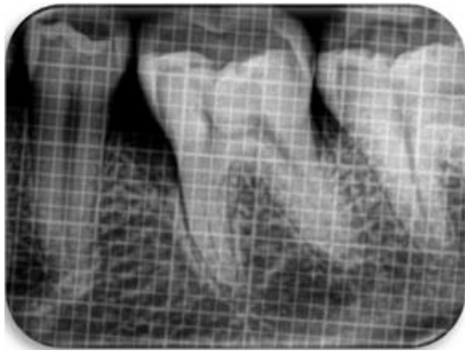

Figure 6: Measurement of intrabony defect fill at 6 months

Table 1: Clinical and radiographic parameters in both the at baseline, 3 months and 6 months

Parameters (mm)	Visit	L - PRF group		A - PRF group	
		Mean +SD	P Value	Mean +SD	P Value
GI	Base line	1.15 ±0.29		1.04 ±0.24	
	3 months	0.71± 0.18	0.0002*	0.73 ±0.13	0.0001*
	6 months	0.50 ±0.10	0.0001*	0.47 ±0.11	0.0001*
PPD	Base line	5.90± 0.81		5.97 ±0.78	
	3 months	4.75± 0.69	0.0001*	4.07 ±0.35	0.0001*
	6 months	4.25 ±0.65	0.0001*	3.53 ±0.30	0.0001*
CAL	Base line	6.42± 0.99		6.18 ±0.82	
	3 months	5.22± 0.74	0.0001*	4.30 ±0.37	0.0001*
	6 months	4.63± 0.75	0.0001*	3.75 ±0.34	0.0001*
IBD	Base line	7.94 ±2.50		9.30 ±1.94	
	3 months	6.93± 2.05	0.0009*	7.02 ±1.22	0.0001*
	6 months	6.30± 1.78	0.0001*	6.02 ±1.02	0.0001*

***p<0.05**

Table 2: Changes (mean SD) in clinical and radiographic parameters between the groups over a 6 - months period

Parameters	Control group	Test group	P value
Gingival index (GI)	0.65±0.25	0.57±0.22	0.454
PPD (mm)	1.65±0.78	2.43±0.69	0.0071*
CAL (mm)	1.78±0.91	2.43±0.67	0.03*
IBD depth reduction (mm)	1.64±1.11	3.28±1.40	0.0014*
Bone defect fill (%)	20.71%	35.27%	0.0001*

***p<0.05**