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Original Research

Comparative evaluation of open flap debridement with and without platelet rich fibrin membrane in treating horizontal bone defects

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ABSTRACT:
Aim: A comparative evaluation of open flap debridement with and without platelet rich fibrin membrane in treating periodontal bone defects. **Material and Methods:** This study was conducted in the Department of Periodontics and Oral Implantology, Maharaja Ganga Singh Dental College and Research Centre, Sri Ganganagar, Rajasthan. A total of 20 patients were selected for relative study consisting of open flap debridement with PRF and open flap debridement without PRF in horizontal bone defects. Group-I: It comprised of 10 individuals to which Kirkland flap procedure was performed with horizontal bone defects. Group-II: It comprised of 10 individuals to which Kirkland flap was performed with PRF membrane in horizontal bone defcts. Gingival index, Plaque index, Probing depth, Clinical attachment level and bone fill were evaluated at baseline, 1month, 2 months and 3 months after surgery. Radiographic assessment was carried out to measure the bone fill at baseline, 1month, 2 months, 3 months after the surgery using radiographic grid. **Result:** The statistical evaluation obtained after 3months showed no significant difference between the groups in relation to Plaque index, Gingival index and Clinical attachment level. Though the mean PPD score decreased in both groups, the values were very similar in both the groups after 1st, 2nd and 3months. Mean bone fill value remained the same in both groups at the end of 1st, 2nd and 3rd months. **Conclusion:** Use of PRF membrane significantly improved the clinical parameters in terms of probing depth, Clinical attachment level and Gingival index but there was no significant radiographic improvement in the bone levels at 3 months post surgery.
Keywords: Horizontal defect, platelet rich fibrin membrane, Kirkland flap

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INTRODUCTION

Periodontal disease is a chronic inflammatory disease of periodontium and its advanced form is characterized by periodontal ligament loss and destruction of surrounding alveolar bone.[1] Periodontitis is one of the most common bacterial infections in humans and it is a consequence of destructive host immune responses to pathogenic bacterial species resulting from the dysbiosis of oral microbiota.[2] Removal or inhibition of subgingival plaque thus plays an important role in maintenance of oral health.[3]Periodontal disease alters the morphologic features of bone in addition to reducing height it leads to various patterns of bone loss of which horizontal and vertical bone loss are the most common bone destruction patterns.[4] The most common pattern of bone loss experienced is horizontal bone loss which is also called a zero wall defect as stated by Kern et al. 1984.It was observed that vertical bone loss with prevalence of (7.8) received (96.8) treatment options whereas horizontal bone loss, with an overwhelming prevalence (92.2), received only(3.2) treatment modalities.[5] In aspect of horizontal defects, open flap debridement has obtained fame in the literature.[6]

Horizontal pattern of bone loss in periodontal disease is the most common type in which the height of the bone is reduced with margin of bone roughly remaining perpendicular to surface of Historically, periodontal surgery was used to treat patients with generalized disease and because of this the surgical approach was usually designed to treat multiple

adjacent teeth. The goal of periodontal surgery has always been to alleviate or eliminate the degeneration associated with the progressive periodontal disease. In order to accomplish this goal, access to the periodontal defect for debridement has been an integral part of surgical therapy.[8]

Periodontal flaps for access provide a means to reduce residual calculus, especially more efficient even in deeper pockets, both in anterior and posterior teeth.[9] Surgical access has shown to improve the efficiency of scaling and root planing[10].

Ideally, periodontal therapy should eliminate inflammation, arrest progression of periodontal disease, improve esthetics, and create an environment conductive to maintenance of health.[11]It involves controlling of periodontal infection and aims at regeneration of the lost periodontium[12].

Many of these procedures include the use of bone grafts and bone replacement materials.[13] Attempts to improve horizontal bone level have also been made with DFDBA in particle, strut and laminar forms in combination with GTR.[14] Enamel matrix protein (EMP) and recombinant human bone morphogenic protein (rhBMP) have also been tested for the treatment of horizontal defect.[15] Kotchy and Laky[16] acknowledged the fact when they stated, ' there is no evidence in the literature of attempts at regenerating lost alveolar bone supracrastally.' Improvement of alveolar bone level by use of systemic antibiotics,[17] anti-inflammatory drugs,[18] bisphosphonates,[19] distraction osteogenesis,[20] rhBMP-2[21] has been attempted but with mixed and sometimes, discouraging results.

Recently, various growth factors have been studied in periodontal regenration[22] and it is indicated that they might strongly alter the healing process[23]. Platelet rich fibrin (PRF) described by Choukroun et al.[24] is a second generation platelet concentrate which contains platelets and growth factors in the form of fibrin membranes prepared from the patient's own blood free of any anticoagulant or other artificial biochemical modifications. It forms a strong fibrin matrix with a complex of three dimensional architecture, in which most of the platelets and leucocytes from the harvested blood are concentrated.[25] Platelet rich fibrin membranes can be used in various regenerative treatments[26] to accelerate healing, to progress the regeneration process, and also as a scaffold in tissue engineering. According to Choukron et al. PRF was initially used in implant surgery to enhance the healing properties of bone.[30] According to Chang et al. PRF promotes the expression of phosphorylated extracellular signal-regulated protein kinase (p-ERK) and stimulates the production of osteoprotegrin (OPG) which inturn causes proliferation of osteoblasts.[31] Another study by Huang et al. reported that PRF stimulates the osteogenic differentiation of the human dental pulp cells by upregulating osteoprotegrin and alkaline phosphatase expression.[32]

The main disadvantage of PRF is its storage after prepration.[33] so it should be used immediately after prepration as it will shrink resulting in dehydration altering the structural integrity of PRF. Dehydration also results in decreased growth factor content in PRF[34] and leukocyte viability will be adversely affected altering its biological properties.

It is a healing biomaterial with tremendous potential for bone and soft tissue regeneration.[35]Platelet rich fibrin membrane can be used alone or in combination with various graft materials. It promotes healing of wound, osseous growth, graft stabilization and hemostasis. It also helps in improving the handling property of bone grafting material.[36]

In view of this, study carried out for evaluating the role of PRF membrane in open flap debridement for treatment of periodontal horizontal bone defects.

MATERIAL AND METHODS

STUDY DESIGN

This study was conducted in the Department of Periodontics and Oral Implantology, Maharaja Ganga Singh Dental College and Research Centre, Sri Ganganagar, Rajasthan. A total of 20 patients were selected for relative study consisting of open flap debridement with PRF and open flap debridement without PRF in horizontal bone defects. Patients received the verbal explanation of the nature of the study and informed written consent was obtained. It was approved by the Medical Ethical Committee of Maharaja Ganga Singh Dental College and Research Centre, Sri Ganganagar, Rajasthan.

CRITERIA FOR PATIENT SELECTION

For standardization of sample, patients were selected on the basis of following criteria.

INCLUSION CRITERIA

- Chronic periodontitis patients with probing depth ranging from 5 to 8mm.
- Subjects aged between 20-55 year with horizontal bone defect.
- Systemically healthy patients.

EXCLUSION CRITERIA

- Patients with known systemic illness.
- Pregnant or lactating mothers.
- Patients with history of allergies to drugs and use of antibiotics within previous 6 months.
- Subject with habit of smoking and tobacco chewing.
- History of periodontal surgery in same area within 6 months.

STUDY PROTOCOL

A proforma was prepared for the study so as to have systemic recording of all observations & information. Clinical parameters like Probing depth (PD), Gingival index and clinical attachment level was measured under standard conditions of light using

mouth mirror & UNC-15 probe . After completion of initial periodontal treatment including oral hygiene instructions, SRP (scaling and root planing) the defects were analysed clinically & radiographically & therefore were scheduled for surgery.

OBTAINING PRF

5ml of peripheral venous blood was drawn from antecubital fossa of the right arm and placed in sterilized vacuum evacuated vials without an anticoagulant and centrifuged immediately for 10 mins at 3000 rpm.

After centrifugation the resultant product,

1. Top most layer, consisted of straw colored acellular plasma
2. The middle layer consisted of PRF clot.
3. Third layer formed was red colored lower fraction containing red blood cells.

The middle layer of PRF clot was then removed with sterile tweezer and separated from the underlying RBC layer using scissors and then transferred on a sterile dish. The obtained PRF was then filled in the kirkland flap.

PRF has an intimate assembly of cytokines, glycans chains and structural glycoproteins, which are enmeshed within a slowly polymerized fibrin network; it has the potential to accelerate soft and hard tissue healing.

STUDY DURATION

3 months

CLINICAL PARAMETERS ASSESSED

The following clinical parameters was assessed and recorded to the nearest mm.

- Gingival index – LOE & SILNESS (1963)
- Plaque index – SILNESS & LOE (1964)

USING UNC-15 PROBE

parameters measured are:

- Probing pocket depth
- Clinical attachment level
- Bone fill

Group-A It comprised of 10 individuals to which Kirkland flap procedure was performed with horizontal bone defects.

Group-B Itcomprised of 10 individuals to which Kirkland flap was performed with PRF membrane in horizontal bone defects.

MATERIALS

- Mouth mirror
- Periodontal probe
- Tweezer
- Syringe-5ml
- Needle
- Cotton
- Sterile tube
- Tweezer
- Sterile tube
- PRF
- Centrifugal machine

RADIOGRAPHIC ASSESSMENT (1MM RADIOGRAPHIC GRID)

There are ample modern diagnostic imaging tools available, but the affordability, availability and radiation exposure remains the concern. Intraoral periapical radiographs (IOPAR) are widely used for the preoperative planning and evaluation for most minor oral surgical procedures owing to it simplicity, significantly lower cost, less radiation exposure and easy availability in a dental clinical set-up. Using these radiographs with a grid aids in increasing the accuracy of the linear measurements for the treatment planning. A radio-opaque metal mesh or a grid can be placed between the object/structures to be imaged and the radiographic film/sensor at the time of x-ray exposure. The two adjacent parallel lines of the grid used should be equidistant.

- The linear distances in two dimensions can be measured for pre-surgical planning using the following mathematical formula**:**
- Actual distance between two points (grid) /Measured distance between two points (grid) ¼ Actual distance between two points(anatomic) /Measured distance between two points (anatomic).

METHOD

- **Preoperative assessment** was performed using intraoral periapical radiograph (IOPAR) or orthopantomogram (OPG) to assess the bony defects and to exclude any pathology like cyst, tumor, etc.
- Routine haemotological assessment was performed for all subjects.

- **PRF PREPARATION**

The PRF was prepared following the protocol developed by Choukroun et al. and used in their previous study. Just before surgery, intravenous blood (by veni puncturing the antecubital vein) was collected in 10-mL sterile tubes without anticoagulant and immediately centrifuged in a centrifugation machine at 3,000 revolutions (400 g) per minute for10 minutes. Blood centrifugation immediately after collection allowed for the composition of a structured fibrin clot in the middle of the tube just between the red corpuscles at the bottom and acellular plasma (platelet-poor plasma [PPP]) at the top. The PRF was easily separated from the red corpuscule base (preserving a small red blood cell [RBC] layer) using sterile tweezers and scissors just after the removal of PPP and then transferred onto a sterile compress. A stable fibrin membrane was obtained by squeezing serum out of the PRF clot.

➢ **SURGICAL PROCEDURE**

An intraoral antisepsis was performed with a 0.12% chlorhexidine digluconate rinse, and an iodine solution was used to carry out an extraoral antisepsis. After the administration of local anesthesia, buccal and lingual crevicular incisions were made, and mucoperiosteal flaps were reflected. Care was taken to preserve as much of the interproximal soft tissue as possible. Meticulous defect debridement and root planing were carried out using ultrasonic instruments and area-specific curettes. No osseous recontouring was carried out.

In GROUP I; 10 patients with horizontal defect in which open flap debridement will be performed.

In GROUP II ; 10 patients with horizontal defect in which open flap debridement will be performed with PRF.

➢ The mucoperiosteal flaps were repositioned and secured in place using a 3-0 non-absorbable silk surgical suture. Interrupted sutures were placed. The surgical area was protected and covered with a periodontal dressing.

Fig 1: Armamentarium

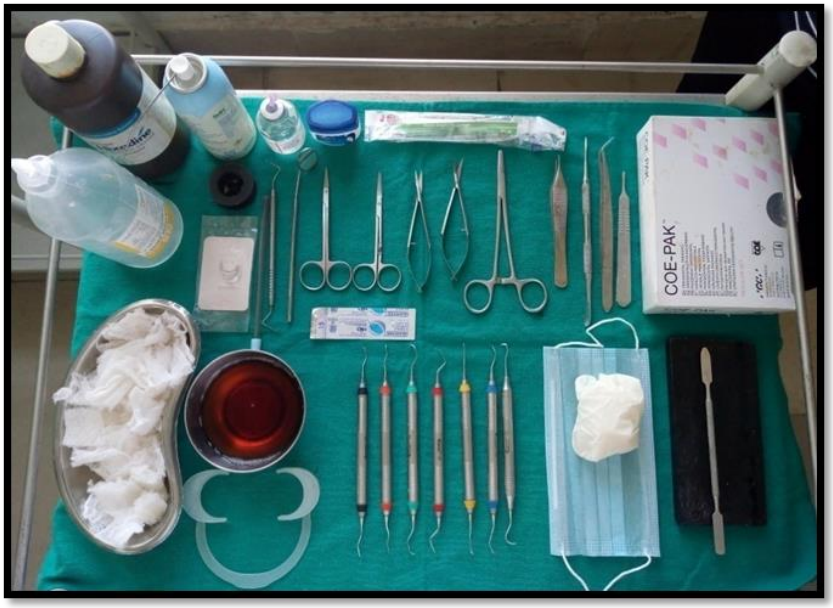
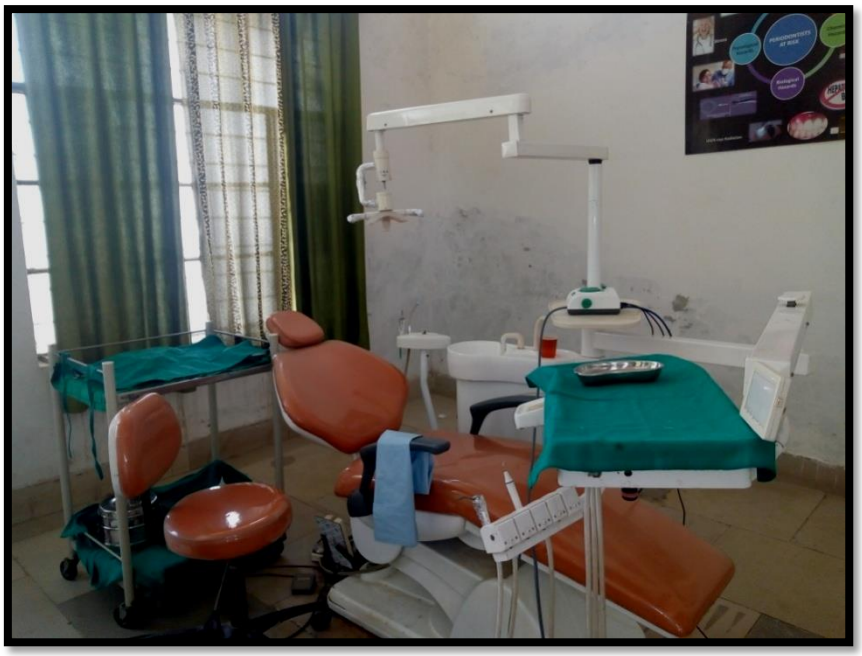
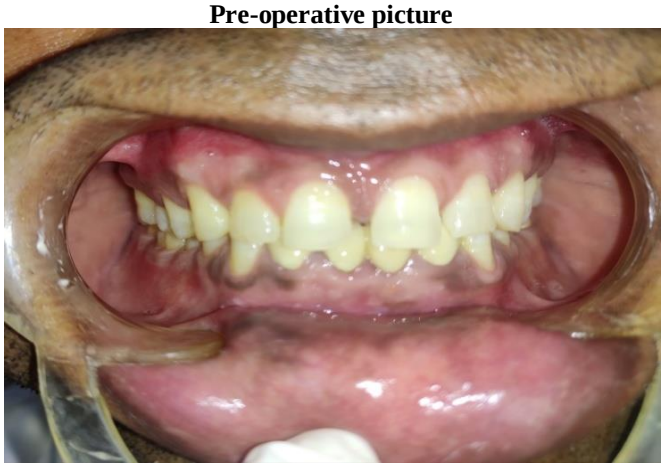


Fig: Dental Operatory Tool

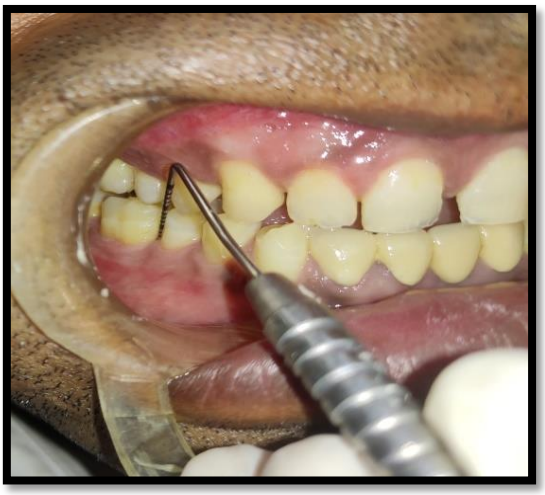


Group I- Subject having horizontal bone defect treated with Kirkland flap

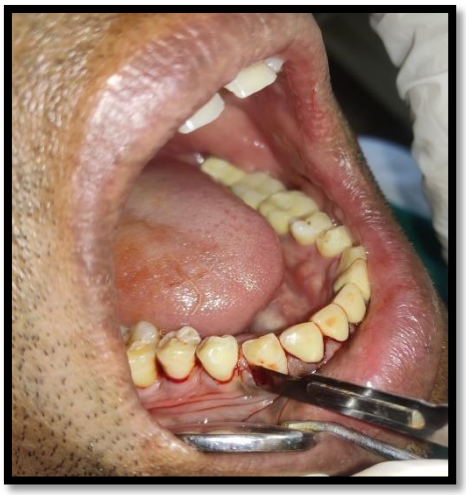
Pre-operative picture



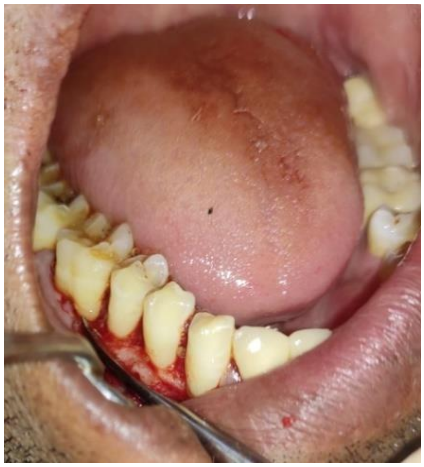
Probing depth



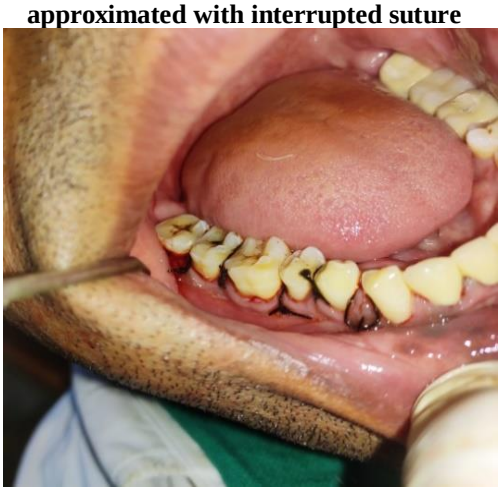
Crevicular incision



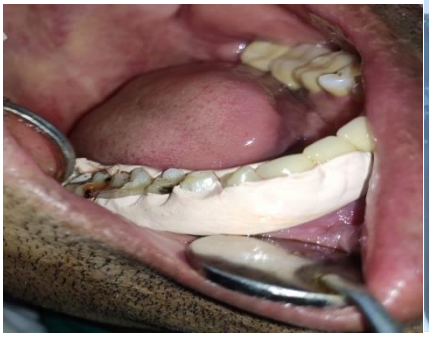
Flap elevation and degranulation done



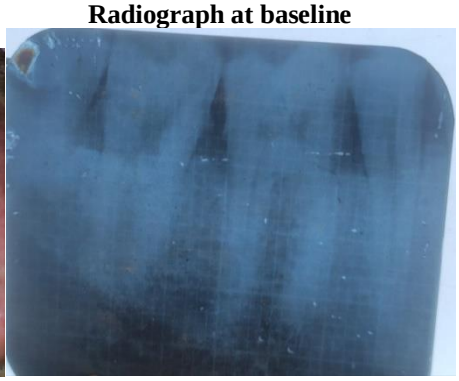
Flap was positioned back and approximated with interrupted suture



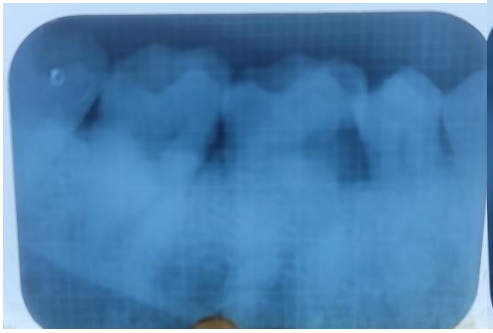
Copak placed



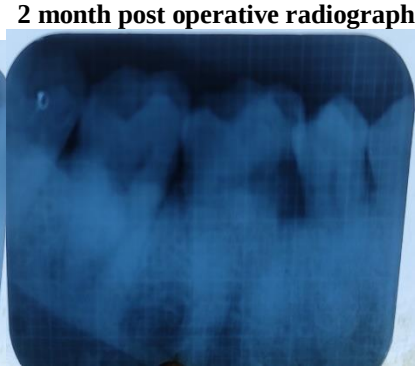
Radiograph at baseline



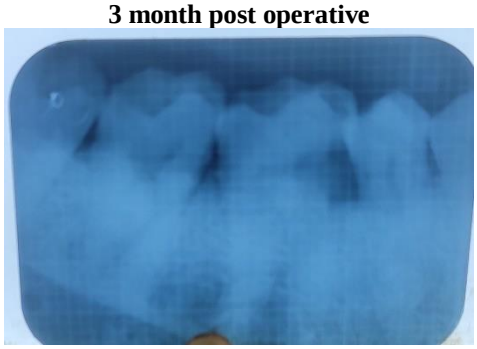
1 month post operative radiograph



2 month post operative radiograph



3 month post operative



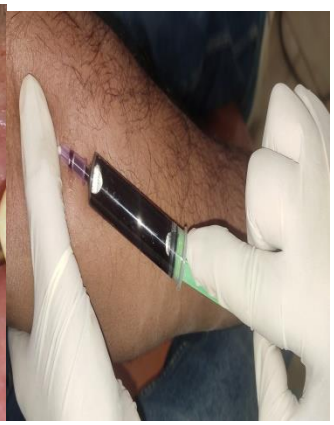
Group II – Subject having horizontal bone defect treated with Kirkland flap and PRF membrane

Pre operative picture

Crevicular incision

Flap elevation and degranulation done

Blood withdrawn



Centrifuge machine

PRF membrane

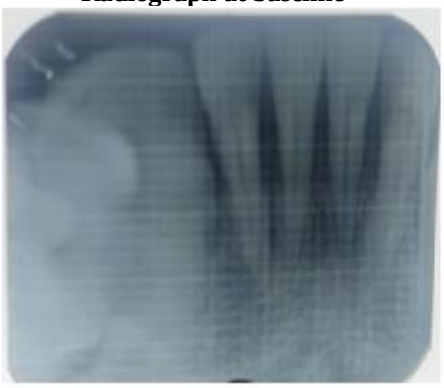
PRF membrane

PRF membrane placement

Interrupted Sutures

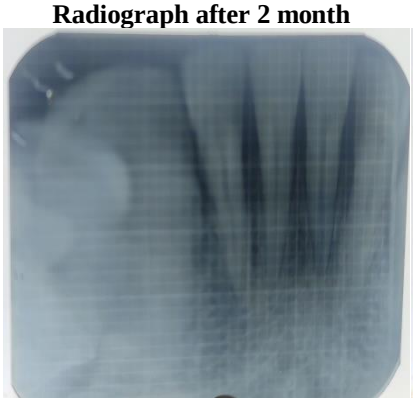
Copak placed

Radiograph at baseline

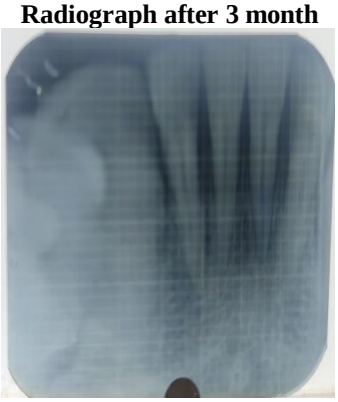


Radiograph after 1 month

Radiograph after 2 month



Radiograph after 3 month



POSTOPERATIVE CARE

Suitable antibiotics and analgesics (500 mg amoxicillin+125 mg clavulanate, three times per day; for 8days and 400 mg ibuprofen, three times per day, for 5 days) were prescribed along with chlorhexidine digluconate rinses (0.12%) twice daily for 2 weeks. Periodontal dressing and sutures were removed 1 week postoperatively. Surgical wound were gently cleansed with povidine iodine, and patients were instructed regarding gentle brushing with a soft toothbrush. Each patient was instructed regarding proper oral hygiene measures postoperatively.

The clinical parameters will be measured from baseline and later during follow up visits at I month, 2 months and 3 months. No subgingival instrumentation was attempted at any of these appointments.

RESULTS

PLAQUE INDEX

Graph 1: Comparison of the means of PI between the group I and group II at baseline, 1st month, 2nd month and 3rd month

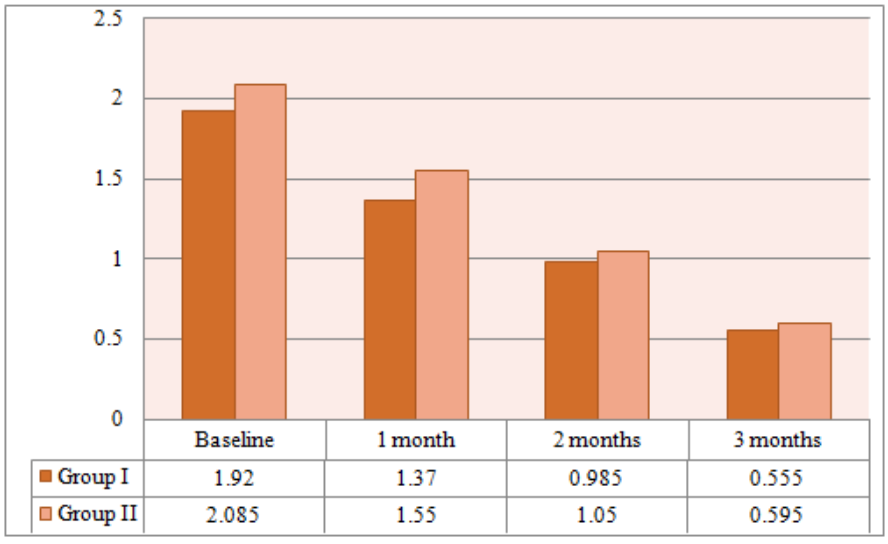


Table 1 Comparison of the means of PI between the group I and group II at baseline, 1st month, 2nd month and 3rd month.

PI		Baseline	1 month	2 months	3 months
Group I	**Mean**	1.920	1.370	.985	.555
	Std. Deviation	.5412	.4715	.3816	.2266
Group II	**Mean**	2.085	1.550	1.050	.595
	Std. Deviation	.4448	.4223	.4282	.2397
p-value		.912[a]	.353	.724	.706

Table 1 represents the comparison of means of plaque index score between group I and group II at baseline, 1st month, 2nd month and 3rd month. The mean plaque index score was found to decrease in both the groups. In group I, the mean score decreased from 1.920 to 0.555 while in group II it decreased from 2.085 to 0.595 at the end of three months. No statistically significant difference was observed between the groups after 1st, 2nd and 3rd month of follow up.

GINGIVAL INDEX

Graph 2: Comparison of the means of GI between the group I and group II at baseline, 1st month, 2nd month and 3rd month

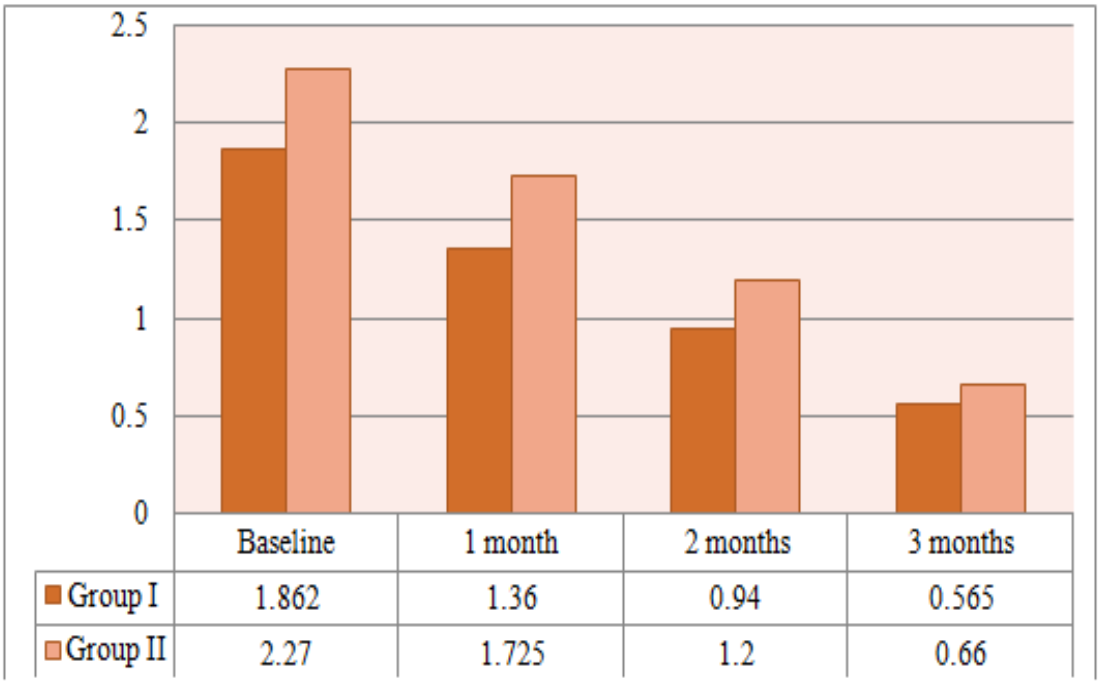


Table 2: Comparison of the means of GI between the group I and group II at baseline, 1st month, 2nd month and 3rd month.

GI		Baseline	1 month	2 months	3 months
Group I	**Mean**	1.862	1.360	.940	.565
	Std. Deviation	.6369	.4142	.3438	.2028
Group II	**Mean**	2.270	1.725	1.200	.660
	Std. Deviation	.4270	.4686	.3801	.2826
p-value		.110	.081	.126	.399

Table 2 shows the comparison of means of gingival index scores between group I and group II at baseline, 1st month, 2nd month and 3rd month. The mean gingival index score revealed a declining trend in both the groups with increasing duration of time. However no statistically significant difference existed between the groups after 1,2 or 3 months.

PROBING POCKET DEPTH

Graph 3: Comparison of the means of PPD between the group I and group II at baseline, 1st month, 2nd month and 3rd month

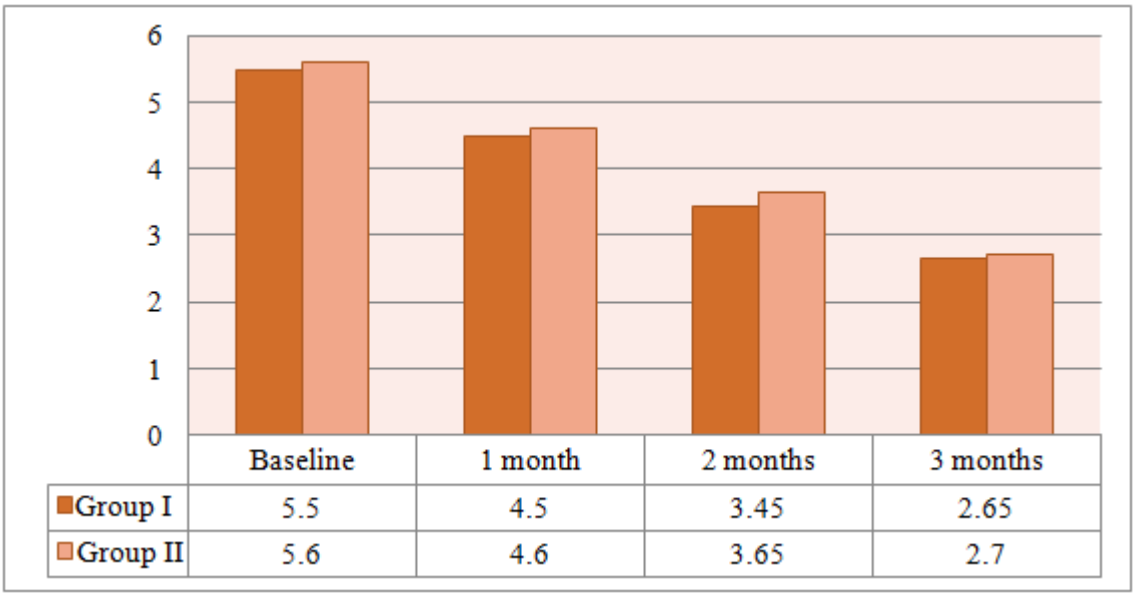


Table 3 Comparison of the means of PPD between the group I and group II at baseline, 1stmonth, 2nd month and 3rd month.

PPD		Baseline	1 month	2 months	3 months
Group I	**Mean**	5.50	4.50	3.45	2.65
	Std. Deviation	.707	.667	.685	.709
Group II	**Mean**	5.60	4.60	3.65	2.70
	Std. Deviation	.516	.516	.580	.537
p-value		.631	.579	.436	.739

Table 3 represents the comparison of the means of PPD between the group I and group II at baseline,1st month, 2nd month and 3rd month. Though the mean PPD score decreased in both the groups, the values were very similar in both the groups after 1st,2nd and 3rd month.

CLINICAL ATTACHMENT LEVEL

Graph 4: Comparison of the means of CAL between the group I and group II at baseline, 1stmonth, 2nd month and 3rd month

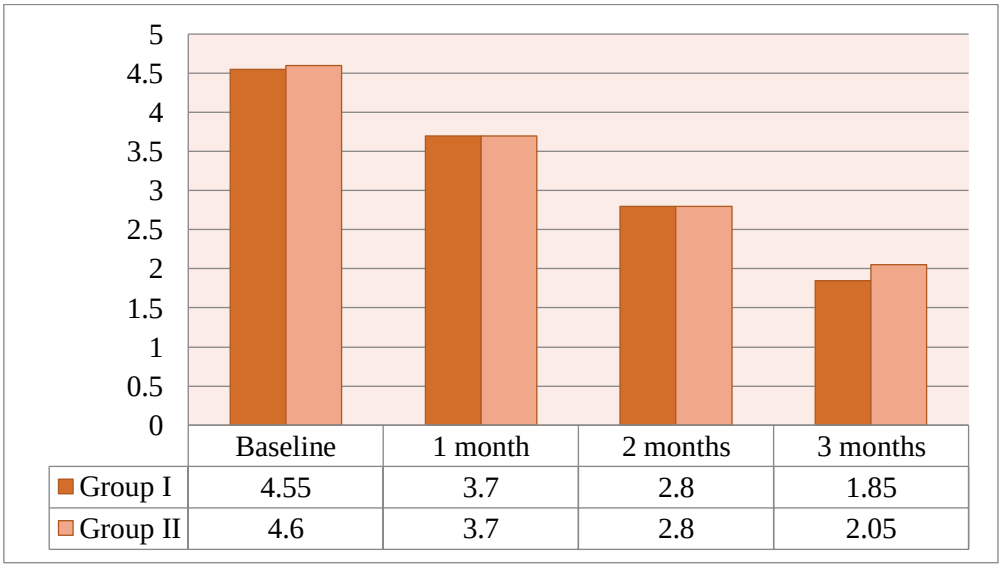


Table 4 Comparison of the means of CAL between the group I and group II at baseline, 1st month, 2nd month and 3rd month.

CAL		Baseline	1 month	2 months	3 months
Group I	Mean	4.550	3.700	2.800	1.850
	Std. Deviation	.6852	.7528	.6749	.5798
Group II	Mean	4.600	3.700	2.800	2.050
	Std. Deviation	.5164	.6325	5375	.4972
p-value		.739	1.000	1.000	.418

Table 4 shows comparison of the means of CAL between the group I and group II at baseline, 1st month, 2nd month and 3rd month. After 3 months, the mean clinical attachment level in group I was 1.850 ± 0.58 while in group II it was 2.050 ± 0.50. No significant difference was observed between groups at all three follow up time periods.

BONE FILL

Graph 5: Comparison of the means of bone fill between the group I and group II at baseline,1st month, 2nd month and 3rd month

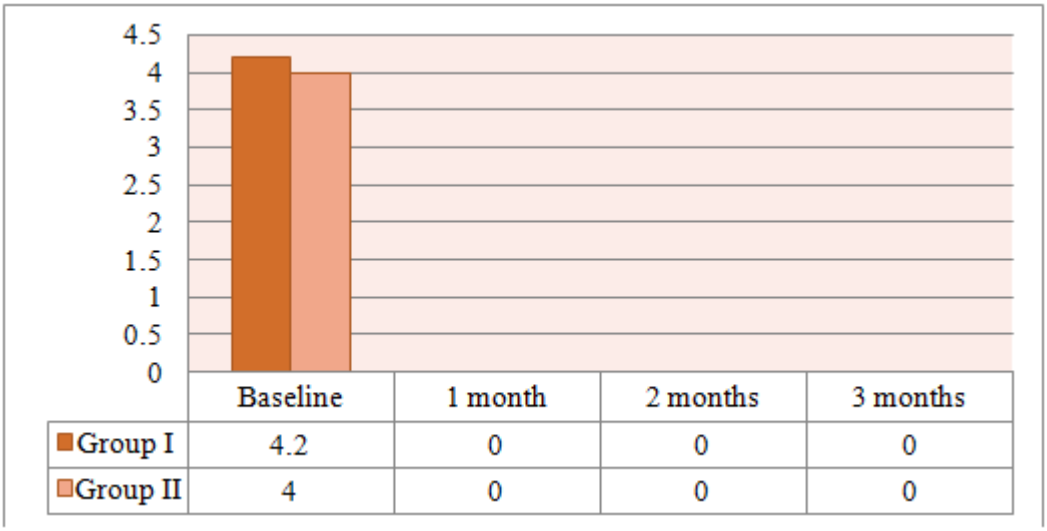


Table 5 Comparison of the means of bone fill between the group I and group II at baseline, 1st month, 2nd month and 3rd month.

Bone fill		Baseline	1 month	2 months	3 months
Group I	Mean	4.20	.00	.00	.00
	Std. Deviation	1.033	.000	.000	.000
Group II	Mean	4.00	.00	.00	.00
	Std. Deviation	.816	.000	.000	.000
p-value		.739	-	-	-

Table 5 shows comparison of the means of bone fill between group I and group II at baseline,1st month, 2nd month and 3rd month. Mean bone fill value remained the same in both groups at the end of 1st,2nd and 3rd month.

DISCUSSION

The aim of periodontal therapy is to remove the destructed inflamed tissues and regain healthy periodontal tissue[37] and to preserve, improve and maintain the natural dentition.[38]Non surgical therapy for the control of periodontitis normally consist of subgingival debridement combined with oral hygiene instructions. Subgingival debridement in the absence of adequate oral hygiene measure results in a limited healing response.[39]Non surgical therapy is the cornerstone of periodontal therapy and the first recommended approach to the control of periodontal infections and it is also known as 'cause related therapy'[40], 'Phase I therapy or 'Etiotrophicphase'andinitial therapy'. Although nonsurgical periodontal therapy has evolved over the years, it is still considered to be the 'gold standard' to which other treatment methods are compared.[41,42]

Kho et al .[43] reported that with supragingival scaling showed no significant changes in the subgingival bacterial composition of pockets with 7mm or deeper.

In contrast**, McNabb et al[44]** reported that supragingival plaque control by professional tooth cleaning induced significant changes in the composition of subgingival microflora, including a

decrease of Porphyromonas gingival is and spirochetes.

Haffjee et al.[45]reported reduction in gingival redness, bleeding reduction of 40 bacterial species including Aggregatibacteractinomycetemcomitans,
P.gingivalis, Prevotella intermedia, and Treponema denticola and mean gain in attachment level.

Proye et al[46] reported reduction in recession after 1 week and a gain of clinical attachment by 3 weeks after a single episode of SRP. Further more, **Isidor et al**[47] observed no changes in the bone height with horizontal bone loss when treated with non-surgical periodontal therapy and also study done by **Badersten et al**[48] reported reduction of approximately 2mm in pocket depth with single instrumentation and no further improvement was achieved with repeated instrumentation.

Mangnusson et al. reported reduction in means pocket depth after single episode from 7.2mm to 6mm within 16 weeks and a second instrumentation decreased pockets to 4.9mm.[49]The outcome of any treatment method is determined by complete and adequate access to pocket areas, the time devoted by the operator to the procedure, and the thoroughness of the procedure.[5o]

Mehraj et al.[51] in their study found that surgical therapy gave better result that non surgical therapy, due to better access to the root debridement. Also**David et al.**[52] suggested that scaling and root planing did not attain treatment goals, and periodontal surgery should be considered as the next step. Treatment of mild to moderate chronic periodontitis had to focus on establishing excellent patient plaque control and did not have to treated surgically. On the other hand, surgical therapy offered benefits beyond scaling and root planing in treating severe periodontitis. A study by **Hetiz-Mayfield et al.**[53] suggested that chronic periodontitis with pocket depth more then 5mm, open flap debridement gain clinical attachment level significantly than scaling and root planing only.

In periodontitis, the presence of subgingival plaque biofilms in susceptible individuals determines an inflammatory reaction, leading to loss of the supporting connective tissue and alveolar bone. Periodontal osseous destruction can result in horizontal or vertical bony defects, depending on the direction and extent of the apical propagation of the plaque induced lesion.[54]

The overall objective of the treatment is the elimination of periodontal inflammation through disruption of the subgingival film, with reduction of gingival probing pocket depth and clinical attachment loss, resulting in reduced risk of disease progression.[55]

Periodontal regeneration requires a multi-dependent orchestrated sequence of biological events, including cell adhesion, migration, proliferation and differentiation. There are many ways and steps for periodontal tissue regeneration like root surface biomodifications, guided tissue regeneration, tissue grafts and bone grafts.[56] Periodontal regeneration requires regaining all periodontal tissues (epithelial tissue, fibroblasts,periodontal ligament fibers, and osteoblasts).[57]

It has been found that during wound healing , platelets and cytokines aggregate within the fibrin clot and several growth factors are released into tissues from the platelets.[58]The presence of growth factors and cytokines in platelets has great role in wound healing.[59] Platelets secrete fibrin, vitronectine, and fibronectin, which act as a matrix for the connective tissue. All of these indicate that usage of platelets concentrates can play potent role in periodontal tissue regeneration.[60] Platelet concentrates can be divided into two generations; the first is platelet-rich plasma(PRP) while the second is platelet rich fibrin (PRF)[61].

PRF consists mainly of fibrin matrix with a large number of platelets and leukocytes.[62]PRF was first prepared by **Dr. Choukroun** from the own patient blood specimen which was centrifuged without adding any anticoagulants or bovine thrombin or any other jellifying agents. PRF is usually dense, consisting of fibrin tissue, leukocytes, and platelets, which is characterized by releasing growth factors and cytokines at a slow rate during a period of 7 days.[63]

It was first described by **Dr. Joseph Choukroun** in France in **2001** to promote wound healing in implants. Currently, the studies have been focused on the use of an autogenous material called Platelet Rich Fibrin that provides an osteoconductive scaffold along with growth factors to stimulate patients's own cells towards a regenerative response[64].

Platelet rich fibrin is a fibrin matrix in which platelet cytokines, growth factors and cells are trapped and may be released after a certain time and that can serve as a resorbable membrane.[120]Growth factors are released after activation from the platelets trapped within fibrin matrix, and have been shown to stimulate the mitogenic response in the periosteum for bone repair during normal wound healing.[65]

The classical technique for PRF preparation was invented by **Dr. Joseph Choukroun** . It is the current PRF technique authorized by the French Health Ministry in which PRF is prepared without using an anticoagulant during blood harvesting or bovine thrombin during gelling.[66]

For preparation of PRF, blood sample is collected from the patient without anticoagulant using a butterfly needle and 10ml blood collection tubes. After collection of blood, it is immediately centrifuged at a rate of 3000 rpm for 10 minutes. After centrifugation, 3 layers are obtained in the test tube. The topmost layer consisting of acellular PPP (platelet poor plasma), PRF clot in the middle and RBCs at the bottom of the test tube. The middle layer of PRF clot is then removed with sterile tweezers and separated from the underlying RBC layer using

scissors and then transferred on a sterile dish. It is supposed that the junction of PRF to the RBC layer is rich in growth factors and therefore this region is preserved[67].

PRF results from a natural and progressive polymerization which occurs during centrifugation.[68]Because of the absence of an anticoagulant, blood begins to coagulate as soon as it comes in contact with the glass surface. Therefore, for successful prepration of PRF, speedy blood collection and immediate centrifugation, before the clotting cascade is initiated, and is absolutely essential.[69] The slow handling of blood to centrifugation process will result in diffuse polymerization of fibrin leading to the formation of a small blood clot with irregular consistency.[70] Also , PRF membrane can be obtained by squeezing out the liquids present in the fibrin clot. Liquid removal from the PRF fraction can be done through mechanical pressure between gauze layers resulting in a fairly solid , gel- like material that can be used in various clinical applications as a filling material or as suturing membrane.[71]

ADVANTAGES OF PRF

1. Simple and cost effective method of preparation of PRF.
2. Eliminates the use of bovine thrombin and thereby reduces the chances of crossinfection. It has been discovered that the use of bovine thrombin may be associated with the development of antibodies to the factors V, XI and thrombin, resulting in the risk of life threatening coagulopathies.[72]
3. Slow natural polymerization of PRF on contact with glass particles of the test tube results in physiologic thrombin concentration, while in PRP, there is sudden fibrin polymerization depending on the amount of surgical additives (thrombin and calcium chloride).[73]
4. Fine and flexible 3-D structure of PRF more favourable to cytokine enmeshment and cellular migration 3-D network-connected tri-molecular or equilateral junctions in PRF allows the establishment of a fine and flexible fibrin network able to support cytokines enmeshment and cellular migration while 3-D organization of PRP consists of fibrin network condensed tetra molecular or bilateral junctions constituted with strong thrombin concentrations which allows the thickening of fibrin polymers leading to a rigid network, not very favourable to cytokine enmeshment and cellular migration.[74]
5. PRF has supportive effect on immune system[75]
6. PRF helps in hemostasis.[76]
7. A in-vitro study showed that PRF is superior to PRP, considering the expression of alkaline phosphatase and induction of mineralization, caused markedly by release of TGF-beta1 and PDGF-AB.[77]

PRF consists of a fibrin matrix polymerized in a tetra molecular structure with the incorporation of platelets leukocyte and cytokines, and the presence of circulating stem cells [78]. PRF stimulates osteoblasts, gingival fibroblasts, and periodontal ligament cells proliferation as a mitogen. Its molecular structure with low thrombin concentration is an optimal matrix for migration of endothelial cells and fibroblasts. [79]It permits a rapid angiogenesis and an easier remodelling of fibrin.The Leukocytes and key immune cytokines like IL 1β, IL 6, IL 4 and TNF α trapped in PRF give it the anti-infectious effect and lets PRF act as an immune regulation mode [80]. **Dohan et al in 2009** classified PRF into pure PRF (P-PRP) or leucocyte poor PRF and leucocyte-rich PRF (L-PRF).[81]**Leukocyte-Poor Or Pure Platelet-Rich Fibrin (P-PRF) Concentrates.** The Fibrinet PRF membrane (PRFM) kit by Cascade Medical (New Jersey, USA) produces a 'natural' platelet concentrate owing to the absence of bovine thrombin. Only very low amounts of leucocytes are collected owing to the specific separator gel used in the method. However, the platelet collection efficiency is high and the preservation of the platelets during the procedure seems to be acceptable, but studies demonstrating the efficiency of Fibrinet PRFM are not yet available.[82]**Leukocyte-rich PRF (L-PRF) Also named Choukroun's PRF, Advanced PRF (A-PRF), and commonly named PRF Membrane has been d**eveloped by Dohan & Choukroun.[83]In this the PRF Box (Process Ltd., Nice, France) is commercially available to prepare the PRF membrane. The PRF clot is placed on the grid in the PRF box and covered with compressor lid which squeezes out the fluid from the clot. The membranes formed using this method have constant thickness of 1mm which remains hydrated for several hours. The serum exudates are also collected under the grid for further use. The serum exudates expressed from the clot are rich in proteins such as vitronectin. These exudates may be used to hydrate graft materials, rinse the surgical site, and store autologous graft.[84]However, another alternative to obtain a PRF membrane developed by **Raja et al**, [85] is by pressing the clot between two gauzes thereby squeezing out the fluids in the fibrin clot. The same was applied for the present study. Further, **Toffler etal**.[86] showed that the PRF clot can also be slowly compressed in a cylinder in the PRF box with an opposing piston to obtain PRF plugs measuring 1 cm in diameter which can be used in socket preservation procedures.

Besides this, various platelet concentrates and fibrin glues have been created that help in the healing of soft and hard tissue.[87]Many longitudinal studies were[88,89,90] carried out on the difference in the releasing kinetics of PRF compared to PRP (platelet rich plasma). In this the major contributor has been,**He et al**.[91] who studied the expression of alkaline phosphatase (ALP) and induction of mineralization under the effects of PRP and PRF in

vitro. A similar study was carried out by **Saluja et al**.[92]and concluded that the limited potential of PRP to stimulate bone regeneration is due to its quick release of growth factors, just before the cell outgrowth and population occurs from the surrounding tissue. **Hatakeyama et al** observed that there were abundant osteogenic cells in PRF group, more than PPP and PRP groups.

PRF matrix can release various growth factors and cytokines locally at the wound site for a prolonged period of time which play important role in various stages of wound healing promoting periapical tissue generation. Growth factors are released from the alpha-granules in the platelets when they are activated, secreted, or aggregated by collagen or epinephrine [93]. TGF-beta and PDGF are the typical two growth factors which promote healing of soft tissue and bone through stimulation of collagen production to improve wound strength and initiation of callus formation [94].

Platelet-derived growth factor (PDGF) is a potent activator for cells of mesenchymal origin. It is among the first cells to reach at the wound site. Strayhorn et al suggested that PDGF might act mostly on osteoblastic cell proliferation, exerting most of its effects during the early phases of wound healing [95]. Transforming growth factor Beta-1 (TGF beta-1), an inflammatory regulator, is the most powerful fibrosis agent amongst all cytokines and can induce a massive synthesis of collagen and fibronectin either by fibroblasts or osteoblasts.[96]

The physiologic fibrin matrix of PRF, obtained as the 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. Moreover, the fibrin matrix itself shows mechanical adhesive properties and biologic functions like fibrin glues: it maintains the flap in a high and stable position, enhances neoangiogenesis, reduces necrosis and shrinkage of the flap, and guarantees maximal root coverage. It plays an important role in angiogenesis and wound coverage.[97]

Angiogenesis requires an extracellular matrix to allow migration, proliferation and phenotype differentiation of endothelial cells. The angiogenesis property of the fibrin matrix is explained by the 3-dimensional structure of the fibrin gel and the simultaneous action of the cytokines trapped in fibrin meshes [98]. Furthermore, main angiogenesis soluble factors such fibroblast growth factorbasic (FGFb), vascular endothelial growth factor (VEGF), angiopectin and platelet derived growth factor (PDGF) are included in fibrin gel which can bind to fibrin with high affinity.

Fibrin matrix guides the wound coverage affecting the metabolism of fibroblasts and epithelial cells. The epithelial cells around the wound margins lose their basal and apical polarity and produce basal andlateral extensions towards the wound site. These cells then migrate onto the transitory matrix made by fibrinogen, fibronectin, tenascin and vitronectin [99]. PRF also aids in trapping circulating stem cells brought to the wound site due to initial neovascularization during hemostasis and healing.[100]

PRF also releases growth factors such as platelet-derived growth factor and transforming growth factor which promote periodontal regeneration .[101] Many studies in past reported that PRF can promote the healing of osseous defects[102-109] As per **Chang et al**.[102] it promotes the expression of phosphorylated extracellular signal-regulated protein kinase (p-ERK) and stimulates the production of osteoprotegerin (OPG)which causes proliferation of osteoblasts.[102] **Huang et al**.[103] reported that it stimulates the osteogenic differentiation of the human dental pulp cells by up-regulating osteoprotegerin and alkaline phosphatase expression.[103]It also releases growth factors such as PDGF and TGF which promote periodontal regeneration.[103,104]**Chang et al[103]** reported that it stimulates cell proliferation in a specific manner. **FurtherTsai et al.**[104] related the regenerative abilities of PRF to the growth factors released by the platelets entrapped within, such as PDGF and TGF in an *in vitro* study. These factors can promote periodontal rgeneration by stimulating specific cell differentiation and proliferation in a specific manner.[104]They stated that PRF had induced cell proliferation of osteoblasts and periodontal ligament cells while suppressing oral epithelial cell growth during a 3-day culture period. These cell type-specific actions thus may be beneficial for periodontal regeneration. **Simon et al.**[105] observed better bone fill of PRF in socket preservation procedure of dog. Similarly**, Bölükbaş et al.** [106] in an *in vitro* study observed more bone formation in PRF and Biphasic calcium phosphate (BCP).

The present study was designed to compared and evaluate the open flap debridement with and without platelet rich fibrin membrane in treating periodontal horizontal bone defects.

In this present study Kirkland flap was performed as given by **Kirkland** in 1931. After raising the flap intra-crevicularly through the bottom of the pocket on both the labial and lingual aspects of the interdental area, root planing and removal of granulation tissue was done.

In group -I only Kirkland was performed and Group -II Kirkland flap was performed and then PRF membrane was placed in defect sites and flaps are sutured.

In this study the mean plaque index score at baseline was 1.920 and mean plaque index scores at 1month, 2month and 3months were 1.370, 0.985, 0.555 for Group I and for Group II at baseline was 2.085 and mean plaque index scores for 1month, 2 months and 3months were 1.550, 1.050 , .595. The mean plaque index found to be decrease in both the groups. The mean plaque index value exhibited at the end of 3months was 0 .706 which was found to be **statistically not significant difference** between inter

groups comparision of the study . This result is in accordance to **Koel Debnath et al 2018** who stated that on intergroup comparision in plaque index also did not shown any statistical evaluation at 9 months follow up period.

Also , the intragroup comparison showed a significant reduction in PPD (probing pocket depth) at the end of 3 months and the mean PPD score resulted was (p=.739) which was found to be statistically non- significant difference when compared between two groups. This result is in accordance to **Rosamma Joseph V et al in 2014**, reported reduction of 0.38mm by open flap debridement only and also reduction in PPD value of 0.45mm in open flap debridement with PRF membrane.

Also, the mean Gingival index score were 1.862, 1.360, .940, .565 at baseline, 1 month, 2 month, and 3months for group-I while for group II were 2.270 , 1.725, 1.200 , .660 at baseline, 1month, 2month and 3 months respectively. The mean gingival index score revealed a declining trend in both the groups with increasing duration of time. However, no statistically significant difference existed between the groups after 1,2 or 3months.

Moreover in present study the mean CAL (clinical attachment level) after 3 months in group I was 1.850 to 0.58 while in group II it was 2.05 to 0.50. No significant difference was observed between groups at all the three follow up time period. The results are in agreement with the study results of **Jane k. Chadwick et al.** suggesting an improved CAL in sites treated using the technique (Open flap debridement+PRF membrane).

In present study, a change of alveolar bone level was detected radiographically consecutive from baseline, 1month, 2months and 3months using intraoral periapical radiographs and radiographic measurements were made utilizing a millimeter X-ray grid, which consisted of vertical and horizontal squares with small squares measuring 1mm. Vertical linear distance between the cementoenamel junction (CEJ) and the most apical extension of the defect were obtained by counting the number of squares of 1mm in a grid.

Therefore, in present study no radiographic defect depth fill was resulted in group I, where open flap debridement was performed in horizontal bone defect and in group II , where open flap debridement along with PRF membrane placement was done for the treatment of horizontal bone defect at the end of 3 months.

PRF is enriched with platelets, growth factors and cytokines increasing the healing potential of both hard and soft tissue.[107]. PRF is considered as a healing biomaterial and is commonly used in implant and plastic periodontal surgery procedures to enhance bone regeneration and soft-tissue wound healing.[108,109] According to **Choukron et al**. PRF was initially used in implant surgery to enhance the healing properties of the bone.[110] PRF when used as a membrane for guided tissue regeneration as a grafting material creates an improved space making effect which facilitates cell events that are favorable for periodontal regeneration leading to mineralized tissue formation. PRF is having an inherent osteoconductive and/or osteoinductive property which is beneficial for regeneration of the bone. **Sanchez et al.** in an experimental study compared the influence of PRP and PRF on proliferation and differentiation of osteoblasts and he reported that the affinity of osteoblasts to the PRF membrane appeared to be superior than the affinity of osteoblasts to PRP.[111]

Post-operatively, antibiotic 500 mg amoxicillin+125 mg clavulanate, three times per day; for 8days were prescribed[112,113]. The combination of amoxicillin and calvulanate makes it resistant to the penicillinase enzyme produced by some bacteria. Generally, systemic antibiotics are generally used as an adjunct to periodontal surgery in specific disease profiles (aggressive/refractory/smokers) [114] for more aggressive treatment, in anticipation and prevention of postsurgical infections. **Bueon and co-worker[115]** reported that 500 mg amoxicillin+125 mg clavulanate arrested alveolar bone loss in patients with periodontal disease. The rationale of using antibiotics with regenerative procedures is to increase the predictability of results by controlling the subgingival microflora in order to reduce the risk of postoperative infection. Studies conducted by **Demolon*et al*[116,117]**and **Loos *et al*.**[118,119]have concluded that use of antibiotic may help to control initial inflammation, but had no direct effects of clinical significance on bone regeneration or soft tissue attachment at 12 months. Ibuprofen 800 mg, three times per day, for 5 days was prescribed to reduce pain, swelling, and to improve wound healing and the treatment outcomes. No post-operative complications were reported in the study.

All patients were given topical chlorhexidine 0.12%(CHX) postoperatively. It enhances wound healing, reduces complications and improves clinical parameters following periodontal surgery. [120,121]Although, it is not possible to determine to what extent and under what conditions CHX may have contributed to reduction in infection, however studies [122,123] confirm the predictability of post surgical control of microflora.

Hence, the results from the present study indicate that open flap debridement alone and with placement of PRF shows significantly improved clinical parameters but no radiographic bone fill in both the groups.

CONCLUSION

In recent years, researchers have focused on biological mediators which have the ability to enhance wound healing and improve clinical benefits of bone replacement grafts. Polypeptide growth

factors are biological mediators which regulate cell proliferation, chemotaxis and differentiation and induce periodontal regeneration.

To deliver high concentrations of polypeptide growth factors to periodontal surgical wounds, use of autologous platelet concentrates serve as a safe and appropriate approach. Various platelet concentrates have been developed to improve soft and hard tissue healing. Platelet rich fibrin is a second generation platelet concentrate, defined as autologous leukocyte and platelet rich fibrin because it collects leukocytes and platelets with high efficiency.

Choukran's PRF is cost-effective and it is obtained through a simplified process.

Kirkland flap along with PRF may be clinically useful in periodontal regeneration supporting the healing process in patients with horizontal bone defect. PRF has thus confirmed itself to be considered as a healing biomaterial.

Use of PRF significantly improved the clinical parameters in terms of porbingdepth , Clinical attachment level and Gingival index but there was no significant radiographic improvement in the bone levels at 3months postsurgery.

Horizontal defect being the most prevalent form of periodontal defect demands more attention by researchers and the use of autologous growth factor delivery system in the form of PRF offers a new dimension in their management.

It is suggested that in future a histological study evaluating the role of PRF in Kirkland flap cases may be carried out to conclusively confirm periodontal regeneration aspects of the same.

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