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Evaluating the efficacy of the combination of 1% metformin gel with platelet- rich fibrin in the management of periodontal intrabony defects – A clinic- radiographic study

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Abstract

Aim: The purpose of the study is to compare clinically and radiographically the efficacy of the combination of 1% Metformin gel (MF gel) with platelet-rich fibrin (PRF) and platelet-rich fibrin alone in the management of periodontal intrabony defects.

Methodology: A split-mouth study was conducted on 15 patients with forty-five surgical sites and was divided into three groups. Group I patients was treated by open flap debridement (OFD) with PRF + 1% Metformin placement, group II patients were treated by OFD with the placement of PRF, and group III patients were treated by OFD alone. Clinical parameters include Plaque index (PI), Gingival index (GI), Probing pocket depth (PPD), Clinical attachment level (CAL), gingival recession (GR), and Russell's periodontal index were recorded at baseline and 3 months and 6 months postoperatively. The depth of the defect was evaluated at baseline, 3 months, and 6 months with the help of a radiographic grid.

Statistical analysis: The intergroup comparisons were performed using a one-way ANOVA test followed by Tukey's Post Hoc test. The intragroup comparison was

performed using repeated measures of ANOVA test followed by Bonferroni's post hoc analysis.

Result: Significant probing pocket depth (PPD) reduction, clinical attachment level (CAL) gain, significant change in Gingival Recession (GR), and a significant reduction in the depth of the defect (DOD) were observed in both groups I and II.

Conclusion: The use of either PRF in combination with 1% metformin or PRF alone was effective in the treatment of intrabony defects with uneventful healing of the sites. The PRF in combination with 1% metformin and PRF appear to have nearly comparable effects, with PRF in combination with 1% metformin displaying slightly superior efficacy in comparison to PRF, which in turn displayed a superior efficacy in comparison to open flap debridement alone.

Keywords: Gingival recession; Growth factors; Metformin; Periodontitis; Periodontal pocket; Platelet-rich fibrin (PRF).

Introduction

Periodontitis is defined as "an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both." Periodontitis demonstrates a clinically detectable attachment loss, usually accompanied by periodontal pocket formation and changes in the density and height of the alveolar bone [1]. Periodontal therapy includes both surgical and non-surgical techniques to restore diseased tissues to a healthy state to prevent tooth loss. Surgical periodontal therapy is incorporated for vertical defects, including bone grafts, membranes like GTR, PRF, etc. [2].

Platelet-rich fibrin (PRF) is a natural fibrin matrix containing platelets and growth factors in fibrin membranes that serve as a resorbable material, developed by Choukroun et al. in France. Thorat et al. from their studies on PRF reported a greater reduction in pocket depth, more gain in clinical attachment level, and greater intrabony defect fill at sites treated with PRF than those treated with open flap debridement alone [3].

Lately, metformin an anti-diabetic agent is used as a local drug delivery agent in patients with chronic periodontitis.

Metformin (1,1-dimethyl biguanide) HCl (MF) is an oral hypoglycemic drug that belongs to second-generation biguanide and is derived from the French lilac (Galega officinalis). The osteogenic effect of MF has been proposed through two mechanisms: an increased proliferation of osteoblasts (Bak et al., 2010, Cortizo et al., 2006) and reduction of osteoclast activity (Liu et al., 2012). Studies have demonstrated that MF downregulates the production of receptor activator of nuclear factor kappa B ligand (RANKL) and up-regulates the production of osteoprotegerin (OPG) from osteoblasts thus, decreasing the osteoclast activity, thereby inducing bone formation and inhibiting bone resorption [4]. Locally delivered MF into the periodontal pocket stimulated a significant increase in the PD reduction, CAL gain, and improved IBD depth reduction compared to placebo in adjunct to SRP (Pradeep A R et al.,2013) [5].

There are very few studies conducted to evaluate the combined effect of PRF and Metformin in intrabony defects using the digital radiographic technique. Hence this study aims to evaluate and compare the efficacy of PRF and the combined effect of PRF and Metformin in periodontal intrabony defects clinically and radiographically using a grid.

Aims and objectives

1. To evaluate clinically the efficacy of Platelet-rich fibrin (PRF) in the treatment of periodontal intraosseous defects.

2. To evaluate clinically the efficacy of the combination of Platelet-rich fibrin (PRF) and Metformin in the treatment of periodontal intraosseous defects.

3. To evaluate the radiographic efficacy of platelet-rich fibrin (PRF) in the treatment of periodontal intraosseous defects.

4. To evaluate the radiographic efficacy of the combination of Platelet-rich fibrin (PRF) and Metformin in the treatment of periodontal intraosseous defects. 5. To compare the clinical and radiographic efficacy of Platelet-rich fibrin (PRF) alone and Platelet-rich fibrin (PRF) and Metformin combined in the treatment of intraosseous defects.

Materials and methods

Study groups: This randomized, longitudinal interventional study involving a total of 15 systemically healthy subjects, contributing to a total of 45 surgical sites, was conducted in our institution. The ethical clearance for the study was obtained from the Institution's Ethical Committee and Review Board. The participants were explained about the study and written consent was obtained from each of the participants.

Patients aged between 35 and 55 years of both sexes, who were systemically healthy and had no contraindications for periodontal therapy were included in the study. Patients diagnosed with periodontitis with clinical attachment loss of 4-6 mm and probing pocket depth of >5mm were included in the study. All the patients were non-smokers.

Patients who were under antibiotics, analgesics, or steroids for over the past 3 months, who received periodontal flap/ regenerative surgery within the past 1 year, pregnant and lactating mothers, platelet count less than 200000/mm3, gingival index score ≥ 2.1 after 2 weeks of phase I therapy, teeth with > Grade II mobility, known/ suspected hypersensitivity to metformin, and taking metformin for any systemic conditions were excluded from the study.

A total of 45 surgical sites were identified and divided into three groups: Group I, Group II, and Group III.

Group I (n= 15): Those to be treated with PRF and 1% MF.

Group II (n=15): Those to be treated with PRF alone.

Group III (n=15): Those to be treated with open flap debridement alone.

Clinical and radiographic assessment

Oral hygiene status was assessed using Plaque Index (Silliness and Loe (1964)) and Gingival Index (Loe and silliness (1963)), Russel's periodontal index (A.L Russel (1956)). PPD, clinical attachment level (CAL), and gingival recession (GR) were measured to the nearest millimeter with a calibrated periodontal probe using an individual occlusal stent as a reference point for probe placement. Occlusal stents for positioning measuring probes were fabricated with cold-cured acrylic resin on a plaster model obtained from an alginate impression.

Measurements were recorded from

- Stent to the cementoenamel junction (A)
- Stent to gingival margin (B)
- Stent to the deepest probing depth at test sites (C).

Calculation of the parameters:

ϖ Probing pocket depth (PPD)= Stent to the deepest probing depth at test sites (C)- Stent to gingival margin (B)

ϖ Clinical Attachment level (CAL)= Stent to the deepest probing depth at test sites (C)- Stent to the CEJ (A)

ϖ Gingival recession= Stent to gingival margin (B)- Stent to the cementoenamel junction (A).

Intraoral periapical radiographs were taken with a radiographic grid in position using the long cone paralleling technique. The depth of the bone defect was assessed on the intraoral periapical radiograph taken with the radiographic grid in position to the closest 0.5 mm. A horizontal line was drawn projecting from the point on the bone crest (A). A horizontal line was drawn perpendicular to the long axis of the root surface of the tooth associated with the vertical defect and the point of contact of the horizontal line with the root surface (B). A vertical line was then drawn from 'B' to the most coronal level along the root surface where the periodontal space was considered to have normal width (C). The vertical dimension between 'B' and 'C' was measured to assess the bone level at the baseline evaluation (BC0).

Preparation of PRF

The PRF was prepared following the protocol developed by Choukroun et al [6] for group I and group II patients. The patient's blood sample was drawn which was transferred to the dried monovettes (without anticoagulant). They were then centrifuged at 3000 rpm for 10 minutes in the tabletop centrifuge. A structured fibrin clot was formed with the red corpuscles at the bottom and acellular plasma at the top (Platelet Poor Plasma-PPP) of the tube. PRF was then separated from red corpuscles and Platelet Poor Plasma (PPP) using a sterile tweezer and scissors, thus preserving a small red blood cell (RBC) layer on the PRF. The PRF obtained was then transferred onto a sterile dappen dish.

Preparation of Metformin

MF gel was prepared as described by the authors in a previous study [5]. The MF gel was developed at the K L E College of Pharmacy, Bangalore, India. All the required ingredients for the formulation were accurately weighed. Dry gellan gum powder was distributed in 50 ml of distilled water maintained at 95°C and was stirred using a magnetic stirrer at 95°C for 20 min. The temperature was maintained above 80°C, and the required amount of mannitol was added to the gellan gum solution and stirred continuously. Metformin was added with stirring. Subsequently, sucralose, citric acid, and preservatives (methylparaben, propylparaben) were added with stirring. Eventually, the required amount of sodium citrate was dissolved in 10 ml of distilled water and added to the mixture. The weight of the gel was monitored continuously during manufacturing and finally, it was adjusted to 100 gm with distilled water. The mixture containing gellan gum, metformin, and other additives was packed in a polyethylene bag with an airtight seal. The gel was formed by allowing the mixture to cool at room temperature. Thus, the MF in situ gel was prepared with a concentration of ~1%.

Transport and storage of metformin gel

The prepared metformin gel was stored in a sterile beaker and covered with aluminum foil and sterilized again. The gel was transported to the college and stored in the refrigerator for future use.

Presurgical procedures

The case history was recorded on a clinical proforma prepared previously, study casts and clinical photographs were taken. Routine lab investigations such as a complete hemogram including platelet count and random blood sugar were done. Phase I therapy which included oral hygiene instructions, scaling, and root planning using hand and ultrasonic instruments were performed. Trauma from occlusion, if present, was relieved. Adjunctive chemical plaque control in the form of Chlorhexidine mouthwash 0.2% twice daily was advised. Patients were re-evaluated 2 weeks following

phase I therapy. Oral hygiene status was assessed using Plaque Index (silliness and Loe (1964)) and Gingival Index (Loe and silliness (1963)).

Surgical procedure

Pre-surgical mouth rinse was carried out with 0.12% chlorhexidine digluconate rinse, which was used to perform intraoral antisepsis. After administration of local anesthesia, sulcular incisions were made both buccally and lingually. Thorough defect debridement and root planning were performed using hand instruments. Presuturing was done prior to the placement of the graft material using a 3-0 non-resorbable braided silk suture. In Group I (OFD + 1% Metformin + PRF), 0.2 ml of 1% metformin and PRF were taken and inserted into the IBD individually. In Group II (OFD + PRF), bone defects were filled with PRF of the required size. In Group III (OFD alone), bone defects were treated with open flap debridement alone. The mucoperiosteal flaps were repositioned and sutured. The surgical area was protected with a non-eugenol periodontal dressing (Coe pack TM, GC America Inc., Chicago, IL, USA). Post-operative instructions and oral hygiene instructions were reinforced.

Post-Surgical Care

Suitable antibiotic and analgesic were prescribed (Tablet Ciprofloxacin (500 mg) + Tinidazole (600 mg) 1-0-1* 5 days and Tab Aceclofenac (100mg) + Paracetamol/Acetaminophen (325mg) + Serratio peptidase (15mg) 1-0-1* 3 days)). Patients were advised to rinse with chlorhexidine digluconate (0.2%) twice a day for 2 weeks following surgery. Patients were advised to avoid smoking. Patients were also advised to avoid brushing the surgical site for 7-10 days. Periodontal dressing and sutures were removed 7-10 days post-surgery. Surgical wounds were cleansed gently with chlorhexidine digluconate of 0.2% and the subjects were informed to brush gently with a soft-bristled toothbrush. Patients were advised to refrain from flossing and using any interdental aids in the area for 4 weeks. Each patient was reinstructed for proper oral hygiene measures at every recall review.

Post-Surgical Evaluation and Review

Gingival Index (GI) and Plaque Index (PI) were reevaluated at 1 month, 3 months, and 6 months. Probing Pocket Depth (PPD), Clinical Attachment Level (CAL), Gingival Recession (GR), and Russel's Index were reassessed at 3 months and 6 months with the formerly used acrylic stents to provide a reproducible insertion axis.

Radiographic parameters

The vertical dimension between "B" and "C" was measured to assess the depth of the defect at 3, and 6. The bone fill at the end of 6 months in each group was obtained by subtracting BC6 from BC0.

Statistical analysis

One-way ANOVA test followed by Tukey's post hoc test was used to compare the mean values of clinical and Radiological parameters between 03 interventional sites during different time intervals.

Repeated Measures of ANOVA test followed by Bonferroni's post hoc analysis were used to compare the mean values of clinical and Radiological parameters between different time intervals in each study site. The level of significance [P-Value] will be set at $P<0.05$.

Results

This randomized longitudinal interventional study was carried out on 15 systemically healthy subjects to evaluate and compare, clinically and radiographically, the efficacy of a combination of Platelet-rich fibrin and 1% Metformin, Platelet-rich fibrin, and open flap debridement alone in the treatment of periodontal end osseous defect. One patient was lost during the follow-

up, thus reducing the total number of study participants to 14. All patients maintained a good level of oral hygiene and gingival status throughout the recall periods.

The subjects included in the study had an age range of 35- 55 years with a mean age of 42.4 years and a standard deviation of 8.007. Of the 15 subjects, 6 were females and 9 were males.

At the end of 6 months, all the groups presented a significant improvement in PPD reduction and CAL gain. The intergroup differences were found to be significant. GR levels had improved in Group II, however, the difference was not statistically significant.

Comparison of Plaque Index Score, and Gingival Index scores in the groups at different time intervals have been shown in Table 1. The plaque index at baseline, 1 month, 3 months and 6 months were 1.38 ± 0.25, 1.02 ± 0.34, 1.10 ± 0.27, and 1.14± 0.14 respectively (Table 4). The mean difference between the values at baseline and 1month, baseline and 3 months, and baseline and 6 months were 0.36, 0.28, and 0.24. The differences were found to be statistically significant. The mean difference between the values at 1 month and 3 months, 1 month and 6 months, and 3 months and 6 months were -0.08, -0.12, and -0.04 respectively. The differences were found to be statistically not significant (Table 5). The gingival index at baseline, 1 month, 3 months and 6 months were 1.30 ± 0.31, 1.06 ± 0.12, 1.05 ± 0.20, and 0.98 ± 0.17 respectively (Table 6). The mean difference between the values at baseline and 1month, baseline and 3 months, and baseline and 6 months were 0.24, 0.25, and 0.32. The differences were found to be statistically significant. The differences were found to be statistically significant. The mean difference between the values at 1 month and 3 months, 1 month and 6 months, and 3 months and 6 months were 0.01, 0.08, and 0.07 respectively. The differences were found to be statistically not significant (Table 7). The Russel's Periodontal Index at baseline, 3 months, and 6 months were 4.51±0.82, 3.861±0.851, and 3.221±0.549 respectively (Table 8). The mean difference between the values at baseline and 3 months, and baseline and 6 months, and 3 months and 6 months were 0.649, 1.289, and 0.64 respectively. The differences were found to be statistically significant (Table 9).

Intergroup comparison (Table 1, 2, and 3):

Probing Pocket Depth

At baseline, there was no statistically significant difference between various groups (P-value =0.66). The difference between Group II and Group III was significant at the end of 3 months (P-value = 0.009); At the end of 6 months, the difference between Group I and Group III, and Group II and Group III were statistically significant (Graph XIV).

Clinical Attachment Level

At baseline, there was no statistically significant difference between various groups (P-value = 0.53). The difference between Group I and Group II, and Group II and Group III were significant at the end of 3 months (Pvalue = 0.002); At the end of 6 months, the difference between Group I and Group II, and Group II and Group III were statistically significant (Graph XV).

Gingival Recession

At baseline, there was no statistically significant difference between various groups (P-value = 0.87). The difference between Group II and Group III was significant at the end of 3 months (P-value = 0.04); At the end of 6 months, the difference between Group I and Group II, and Group II and Group III were statistically significant (Graph XVI).

Depth of the defect

At baseline, there was no statistically significant difference between various groups (P-value = 0.08). The

difference between Group II and Group III was significant at the end of 3 months (P-value = 0.01); At the end of 6 months, the difference between Group II and Group III was statistically significant (P-value = 0.005) (Graph XVII).

Intragroup comparison

Probing pocket depth (Table 10, Graph II, V, and VIII): The Probing Pocket Depth in Group I at baseline, 3, and 6 months were 7.50± 1.51, 5.00± 0.78, and 3.57±0.65 respectively. The mean difference between the values at baseline and 3 months, baseline and 6 months were 2.50, and 3.93 respectively. The Probing Pocket Depth reduced from 7.50± 1.51 to 3.57±0.65 at the end of 6 months. The difference between PPD values at baseline and 3 months, baseline and 6 months, and 3 months and 6 months were statistically significant (P-value <0.001).

The Probing Pocket Depth in Group II at baseline, 3, and 6 months were 7.07± 1.07, 4.57± 0.85, and 3.50±0.76 respectively. The mean difference between the values at baseline and 3 months, baseline and 6 months were 2.50, and 3.93 respectively. The Probing Pocket Depth reduced from 7.07± 1.07 to 3.50±0.76 at the end of 6 months. The difference between PPD values at baseline and 3 months, baseline and 6 months, and 3 months and 6 months were statistically significant (P-value <0.001).

The Probing Pocket Depth in Group II at baseline, 3, and 6 months were 7.07± 1.07, 4.57± 0.85, and 3.50±0.76 respectively. The mean difference between the values at baseline and 3 months, baseline and 6 months were 2.50, and 3.93 respectively. The Probing Pocket Depth reduced from 7.07± 1.07 to 3.50±0.76 at the end of 6 months. The difference between PPD values at baseline and 3 months, baseline and 6 months, and 3 months and 6 months were statistically significant (P-value <0.001).

Clinical attachment level (Table 11, Graph II, V, and VIII): The Clinical Attachment Level in Group I at baseline, 3, and 6 months were 8.21± 1.48, 6.21± 1.12, and 4.93±1.07 respectively. The mean difference between the values at baseline and 3 months, baseline and 6 months were 2, and 3.28 respectively. The Clinical Attachment Level reduced from 8.21± 1.48 to 4.93±1.07 at the end of 6 months. The difference between CAL values at baseline and 3 months, baseline and 6 months, and 3 months and 6 months were statistically significant (P-value ≤0.001). The Clinical Attachment Level in Group II at baseline, 3, and 6 months were 7.79± 0.80, 5.00± 0.96, and 3.64±0.74 respectively. The mean difference between the values at baseline and 3 months, baseline and 6 months were 2.79, and 4.15 respectively. The Clinical Attachment Level reduced from 7.79± 0.80 to 3.64±0.74 at the end of 6 months. The difference between CAL values at baseline and 3 months, baseline and 6 months, and 3 months and 6 months were statistically significant (P-value <0.05). The Clinical Attachment Level in Group III at baseline, 3, and 6 months were 7.64± 1.39, 6.64± 1.74, and 5.79±1.81 respectively. The mean difference between the values at baseline and 3 months, baseline and 6 months were 1.00, and 1.85 respectively. The Clinical Attachment Level reduced from 7.64± 1.39 to 5.79±1.81 at the end of 6 months. The difference between CAL values at baseline and 3 months, baseline and 6 months, and 3 months and 6 months were statistically significant (P-value <0.05).

Gingival recession (Table 12, Graph III, VI, and IX): The Gingival Recession in Group I at baseline, 3, and 6 months were 0.71± 0.83, 1.21± 1.12, and 1.36±1.15 respectively. The mean difference between the values at baseline and 3 months, baseline and 6 months were -0.5, and -0.65 respectively. The Gingival Recession increased from 0.71± 0.83 to 1.36±1.15 at the end of 6

months. The difference between GR values at baseline and 3 months, baseline and 6 months, and 3 months and 6 months were not statistically significant (P-value =0.10). The Gingival Recession in Group II at baseline, 3, and 6 months were 0.57± 0.76, 0.43± 0.65, and 0.14±0.36 respectively. The mean difference between the values at baseline and 3 months, baseline and 6 months were 0.14, and 0.43 respectively. The Gingival Recession reduced from 0.57± 0.76 to 0.14±0.36 at the end of 6 months. The difference between GR values at baseline and 3 months, baseline and 6 months, and 3 months and 6 months were not statistically significant (P-value =0.13). The Gingival Recession in Group III at baseline, 3, and 6 months were 0.57± 0.85, 1.21± 1.19, and 1.36±1.28 respectively. The mean difference between the values at baseline and 3 months, baseline and 6 months were -0.64, and -0.79 respectively. The Gingival Recession increased from 0.57± 0.85 to 1.36±1.28 at the end of 6 months. The difference between GR values at baseline and 3 months, baseline and 6 months were statistically significant (Pvalue <0.05).

Depth of the defect (Table 13, Graph IV, VII, and X):

The depth of defect in Group I at baseline, 3, and 6 months were 4.46 ± 0.93, 2.79 ± 0.78, and 1.89± 0.90 respectively. The mean difference between the values at baseline and 3 months, baseline and 6 months were 1.73, and 2.57 respectively. The depth of defect reduced from 4.46 ± 0.93 to 1.89± 0.90 at the end of 6 months. The difference between the depth of defect values at baseline and 3 months, baseline and 6 months, and 3 months and 6 months were statistically significant (P-value <0.001).

The depth of defect in Group II at baseline, 3, and 6 months were 3.75± 0.47, 2.39± 0.45, and 1.32±0.77 respectively. The mean difference between the values at baseline and 3 months, baseline and 6 months were 1.36, and 2.43 respectively. The depth of defect reduced from 3.75± 0.47 to 1.32±0.77 at the end of 6 months. The difference between the depth of defect values at baseline and 3 months, baseline and 6 months, and 3 months and 6 months were statistically significant (P-value <0.001).

The Gingival Recession in Group III at baseline, 3, and 6 months were 4.04± 0.75, 3.07± 0.62, and 2.36±0.77 respectively. The mean difference between the values at baseline and 3 months, baseline and 6 months were 0.97, and 1.68 respectively. The depth of defect reduced from 4.04± 0.75 to 2.36±0.77 at the end of 6 months. The difference between the depth of defect values at baseline and 3 months, baseline and 6 months were statistically significant (P-value <0.001).

To summarize, the results from the study indicate that Platelet-rich fibrin in combination with 1% Metformin, Platelet-rich fibrin alone, and open flap debridement alone is efficacious in the treatment of periodontal end osseous defects. Platelet-rich fibrin combined with 1% Metformin and Platelet-rich fibrin alone appears to have nearly comparable effects, with platelet-rich fibrin combined with 1% Metformin displaying superior effect in comparison to platelet-rich fibrin. Platelet-rich fibrin combined with 1% Metformin and Platelet-rich fibrin alone has shown a better result in comparison to open flap debridement alone.

Discussion

Periodontal regeneration includes regeneration of alveolar bone, cementum, periodontal ligament, and gingiva. Regenerative periodontal surgery is intended to re-establish periodontal tissues lost as a result of the disease process [7]. Clinically, it may not be clear if the improvement observed is resulted from functional collagenous scar or formation of long junctional epithelium or if the periodontal regeneration has occurred. Therefore, clinical healing may reflect factors

related to a particular procedure while on the contrary, periodontal regeneration is a consequence of biological factors that are active regardless of the protocol [8].

In the present study, only those sites that have shown Interproximal probing depth ≥ 5mm following phase I therapy. In this study, only those sites were included that have shown radiographic evidence of angular bone loss of ≥3mm deep. A Gingival Index score of 2.1 and above after 2 weeks of Phase I therapy was considered in exclusion criteria because it indicated inadequate oral hygiene maintenance by the patient.

Russell's Periodontal Index was assessed at baseline, 3 months, and 6 months using a radiograph with a grid. The results of the study showed a statistically significant decrease in the degree of periodontal disease among various groups at different time intervals suggestive of improved pocket depth reduction and an increase in radiographic bone height.

According to Marx et al, a therapeutic autologous platelet concentrate should present approximately one million platelets per microliter in humans, considering that the whole blood contains approximately 2,00,000 ± 75,000 platelets per microliter [9]. Thus, patients with platelet counts of more than 200000/mm3 were considered vital for the study, and patients with lower platelet counts were excluded from the study.

Probing Pocket Depth (PPD), Clinical Attachment Level (CAL,) and Gingival Recession (GR) were assessed using a UNC 15 probe positioned along the grooves on a customized acrylic stent fabricated for each patient for providing a reproducible insertion axis for the probe.

It has been shown in a study by Payne et al that Probing Pocket Depth (PPD) and Clinical Attachment Level (CAL) measurement reflect changes in the underlying bone level over time and hence are good clinical parameters to assess the potential effects of regenerative materials in the treatment of intrabony defects [10].

Gingival recession (GR) is another clinical parameter that has been considered in the study. Once the periodontal regenerative surgery is done, the next aim is to achieve complete wound healing and regeneration of the periodontal unit. The potential of the various graft materials used in the study to achieve the abovementioned aims mandates the evaluation of their potential effect on gingival recession.

The depth of the defect was assessed using an intraoral periapical radiograph with a grid (IOPA film grid®, BlueDent, Chennai, India). The bone fills were assessed by comparing the IOPAs of 3 months and 6 months with that at baseline. Radiographic grid reduces the inaccuracy behind the manual assessment of bone-fill and the overestimation of bone-fill, and it may be attributed to the enhanced sensitivity of the method [11].

Growth factors promote proliferation (mitogenesis), migration (chemotaxis), and stimulation of new blood vessel formation (angiogenesis), thus favoring wound healing [12]. These naturally occurring molecules are key regulators of these biological events due to the presence of certain matrix proteins. They have been shown to have pleiotropic effects on wound repair, nearly all tissues including the periodontium [13] [14]. PRF when used in combination either with the bone grafts (bovine porous bone mineral, nanocrystalline hydroxyapatite, and demineralized freeze-dried bone allograft [DFDBA]) or pharmacologic agents such as metformin gel, was found to be more effective in terms of improvements in clinical parameters and radiographic defect depth reduction compared to when bone grafts or metformin used alone [15- 19].

PRF an autogenous living biomaterial, developed in France by Choukroun et al. (2001), is a

secondgeneration platelet concentrate. It has gained popularity as it accelerates soft- and hard-tissue healing [20]. A major advantage of PRF is its simple preparation protocol. PRF contains leukocytes and macrophages, known cell types implicated in immunity and host defense [6] [21]. PRF has remarkable regenerative properties for soft and hard tissues without inducing any kind of inflammatory reactions [22]. A study by Kawamura and Urist reported that PRF works as a supportive matrix for carrying morphogenetic proteins [23]. PRF can promote the healing of osseous defects by the following mechanisms. According to Chang et al., PRF promotes the expression of phosphorylated extracellular signal-regulated protein kinase (p-ERK) and stimulates the production of osteoprotegerin (OPG) which in turn causes the proliferation of osteoblasts [24]. PRF releases growth factors such as platelet-derived growth factors and transforming growth factors that promote periodontal regeneration [25, 26]. Metformin (1, 1-dimethyl biguanide) HCl (MF) is a second-generation biguanide, derived from the French lilac (Galega officinalis), used to manage type 2 diabetes mellitus [27]. Metformin promotes osteogenic differentiation of osteoblast-like cells and human chorionic villous mesenchymal stem cells (CV-MSCs) in vitro; an increased bone formation effect has been confirmed in vivo [28- 31]. Metformin is shown to inhibit cytosolic and mitochondrial reactive oxygen species production induced by advanced glycation end products in endothelial and smooth muscle cells [32]. Metformin acts at the cellular level by reducing intracellular reactive oxygen species thus, apoptosis, and exhibits a direct osteogenic effect on osteoblasts. The osteogenic effect is due to insulin-like growth factor-1 expression and is partially mediated via the promotion of Runx2. Further, it can induce MC3T3E1 osteoblastic cell differentiation and bone matrix synthesis through adenosine 5′ monophosphate-activated protein kinase activation and subsequent induction of endothelial nitric oxide synthase and bone morphogenetic protein 2 expressions [33- 35]. An increase in bone fill was demonstrated due to the slow release of growth factors such as insulin-like growth factor 1 expression in the case of metformin, and plateletderived growth factor, transforming growth factor-beta 1 in case of PRF. The release of all these factors has been found to be time-bound in different studies [36]. A study by Borges et al. revealed that a very modest increase in bone mineral density was noted on 80 weeks of treatment with metformin, thus these bone sparing and bone formative effects of metformin may be linked to the passage of time [37]. In this study, 1% MF was used as the 1% concentration of metformin demonstrated to have a sustained release of the drug for 4 weeks when placed as an intrapacket gel [5]. Thus, the growth factors obtained from PRF and Metformin seem to have a wash-off period limiting the accuracy of the study for a period of 6 months [38]. In some studies, metformin has demonstrated delayed wound healing and was found to be independent of the blood glucose levels that have effects on cell proliferation [39, 40].

Group I (PRF +1% MF) showed a greater PD reduction, significant CAL gain, significant bone fill, and increased gingival recession at the end of 6 months. Group II (PRF) showed a greater PD reduction, significant CAL gain, and significant bone fill, at the end of 6 months. Group III (OFD) showed a PD reduction, CAL gain, bone fill, and increased gingival recession at the end of 6 months. The difference among the three groups for the various parameters at the end of 6 months was found to be significant.

The results of the study indicate that on the intergroup comparison, Group I showed a significant decrease in the clinical attachment level when compared to Groups II and III, an increase in gingival recession when compared to Group II, and a significant decrease in pocket depth and depth of the defect when compared to Group III at the end of 6 months.

Thus, the present study concludes that the combination of PRF with 1% metformin showed a comparatively significant improvement in the clinical parameters except for the gingival recession when compared with patients treated PRF alone. Both PRF + 1% Metformin and PRF alone groups showed a significant improvement in the clinical parameters when compared with OFD treated groups.

In the present study, the efficacy of PRF with metformin was assessed for 6 months. The longer evaluative period can aid in interpreting the role of metformin in inducing bone formation.

Limitations of study

The major limitation of the present study is- The small sample size is taken which is inadequate to evaluate the efficacy of graft materials in the treatment of human periodontal intrabony defects. The intrabony defects included in our study differed in their dimension i.e., the width and depth. The treatment outcome is influenced by the differences in the dimensions of the defect. Hence the results of the present study may be influenced by the same.

Conclusion

The following conclusions can be drawn from the study:

1. Clinically, Platelet-Rich-Fibrin (PRF) in combination with 1% Metformin, Platelet-rich Fibrin alone, and open flap debridement alone is efficacious in the treatment of periodontal end osseous defects.

2. Clinically Platelet-Rich-Fibrin (PRF) in combination with 1% Metformin and Platelet-rich fibrin alone appear to have nearly comparable effects in the treatment of periodontal end osseous defects, with Platelet-rich Fibrin in combination with 1% metformin displaying slightly superior effect in comparison to Platelet Rich Fibrin alone. Platelet Rich Fibrin in combination with 1% metformin and Platelet Rich Fibrin has shown a better result in comparison to the open flap debridement alone.

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Table 1: comparison of mean values of different study parameters between 3 groups at baseline period.

Parameter	Groups	N	Mean	SD	P-Value
PI	Group 1	15	1.36	0.26	0.91
	Group 2	15	1.33	0.25	
	Group 3	15	1.36	0.19	
GI	Group 1	15	1.31	0.30	1.00
	Group 2	15	1.31	0.30	
	Group 3	15	1.31	0.30	
PD	Group 1	15	7.67	1.59	0.66
	Group 2	15	7.27	1.28	
	Group 3	15	7.27	1.22	
CAL	Group 1	15	8.33	1.50	0.53
	Group 2	15	7.93	0.96	
	Group 3	15	7.80	1.47	
Gingival Recession	Group 1	15	0.67	0.82	0.87
	Group 2	15	0.53	0.74	
	Group 3	15	0.53	0.83	
Defect Depth	Group 1	15	4.40	0.93	0.08
	Group 2	15	3.73	0.46	
	Group 3	15	4.00	0.73	

Table 2: comparison of mean values of diff. Parameters between 3 groups at 3 months period.

Parameters	Groups	N	Mean	SD	P-Value [a]	Sig. Diff	P-Value [b]
PD	Group 1	15	5.07	0.80	0.01*	G1 vs G2	0.28
	Group 2	15	4.60	0.83		G1 vs G3	0.28
	Group 3	15	5.53	0.83		G2 vs G3	0.009*
CAL	Group 1	15	6.20	1.08	0.002*	G1 vs G2	0.04*
	Group 2	15	5.00	0.93		G1 vs G3	0.50
	Group 3	15	6.73	1.71		G2 vs G3	0.002*
Gingival Recession	Group 1	15	1.13	1.13	0.04*	G1 vs G2	0.12
	Group 2	15	0.40	0.63		G1 vs G3	0.98
	Group 3	15	1.20	1.15		G2 vs G3	0.04*
Defect Depth	Group 1	15	2.73	0.78	0.01*	G1 vs G2	0.25
	Group 2	15	2.37	0.44		G1 vs G3	0.31
	Group 3	15	3.07	0.59		G2 vs G3	0.01*

*Statistically significant

Note: a. P-value derived by One-way ANOVA test.

b. P-value derived by Tukey's Post hoc test.

Table 3: Comparison of mean values of diff. Parameters between 3 groups at 6 months period.

Parameters	Groups	N	Mean	SD	P-Value [a]	Sig. Diff	P-Value [b]
PD	Group 1	14	3.57	0.65	0.005*	G1 vs G2	0.97
	Group 2	14	3.50	0.76		G1 vs G3	0.02*
	Group 3	14	4.43	0.94		G2 vs G3	0.01*
CAL	Group 1	14	4.93	1.07	<0.001*	G1 vs G2	0.03*
	Group 2	14	3.64	0.74		G1 vs G3	0.20
	Group 3	14	5.79	1.81		G2 vs G3	<0.001*
Gingival Recession	Group 1	14	1.36	1.15	0.003*	G1 vs G2	0.008*
	Group 2	14	0.14	0.36		G1 vs G3	1.00
	Group 3	14	1.36	1.28		G2 vs G3	0.008*
Defect Depth	Group 1	14	1.89	0.90	0.007*	G1 vs G2	0.16
	Group 2	14	1.32	0.77		G1 vs G3	0.30
	Group 3	14	2.36	0.77		G2 vs G3	0.005*

- Statistically Significant.

Table 4: Comparison of mean PI values b/w different time intervals.

Time	N	Mean	SD	P-Value
Baseline	14	1.38	0.25	<0.001*
1 Month	14	1.02	0.34	
3 Months	14	1.10	0.27	
6 Months	14	1.14	0.14	

* - Statistically Significant.

Table 5: Multiple comparisons of mean diff. in PI values b/w time intervals.

Time	BL vs 1M	BL vs 3M	BL vs 6M	1M vs 3M	1M vs 6M	3M vs 6M
P-Value	0.001*	0.002*	0.002*	0.14	0.41	0.89

Table 6: Comparison of mean GI values b/w different time intervals.

Time	N	Mean	SD	Min	Max	P-Value [a]
Baseline	14	1.30	0.31	0.9	2.0	0.001*
1 Month	14	1.06	0.12	0.8	1.2	
3 Months	14	1.05	0.20	0.5	1.4	
6 Months	14	0.98	0.17	0.5	1.2	

* - Statistically Significant

Table 7: Multiple comparisons of mean diff. in GI values b/w time intervals.

Time	BL vs 1M	BL vs 3M	BL vs 6M	1M vs 3M	1M vs 6M	3M vs 6M
P-Value	0.004*	0.004*	0.003*	0.57	0.17	0.26

* - Statistically Significant.

Table 8: Comparison of mean Russel's periodontal index (RPI) values b/w different time intervals.

Time	N	Mean	SD	Min	Max	P-Value [a]
Baseline	14	4.510	0.820	3.07	5.74	<0.001*
3 Months	14	3.861	0.851	2.57	5.25	
6 Months	14	3.221	0.549	2.42	4.35	

* - Statistically Significant.

Table 9: Multiple comparisons of mean diff. in RPI values b/w time intervals.

Time	BL vs 3M	BL vs 6M	3M vs 6M
P-Value	<0.001*	<0.001*	0.002*

* - Statistically Significant.

Table 10: Comparison of mean PPD levels between diff. time intervals in each study group.

Groups	Time	N	Mean	SD	P-Value [a]	Sig. Diff	P-Value [b]
Group 1	Baseline	14	7.50	1.51	<0.001*	BL vs 3M	<0.001*
	3 Months	14	5.00	0.78		BL vs 6M	<0.001*
	6 Months	14	3.57	0.65		3M vs 6M	<0.001*
Group 2	Baseline	14	7.07	1.07	<0.001*	BL vs 3M	<0.001*
	3 Months	14	4.57	0.85		BL vs 6M	<0.001*
	6 Months	14	3.50	0.76		3M vs 6M	<0.001*
Group 3	Baseline	14	7.07	1.00	<0.001*	BL vs 3M	<0.001*
	3 Months	14	5.43	0.76		BL vs 6M	<0.001*
	6 Months	14	4.43	0.94		3M vs 6M	<0.001*

* - Statistically Significant.

Note: a. P-Value derived by Repeated Measures of ANOVA Test b. P-Value derived by Bonferroni's Post hoc Test.

Table 11: Comparison of mean CAL levels between diff. time intervals in each study group.

Groups	Time	N	Mean	SD	P-Value [a]	Sig. Diff	P-Value [b]
Group 1	Baseline	14	8.21	1.48	<0.001*	BL vs 3M	<0.001*
	3 Months	14	6.21	1.12		BL vs 6M	<0.001*
	6 Months	14	4.93	1.07		3M vs 6M	0.001*
Group 2	Baseline	14	7.79	0.80	<0.001*	BL vs 3M	<0.001*
	3 Months	14	5.00	0.96		BL vs 6M	<0.001*
	6 Months	14	3.64	0.74		3M vs 6M	<0.001*
Group 3	Baseline	14	7.64	1.39	0.001*	BL vs 3M	0.04*
	3 Months	14	6.64	1.74		BL vs 6M	0.003*
	6 Months	14	5.79	1.81		3M vs 6M	0.003*

* - Statistically Significant

Table 12: Comparison of mean gingival recession (GR) levels between diff. time intervals in each study group.

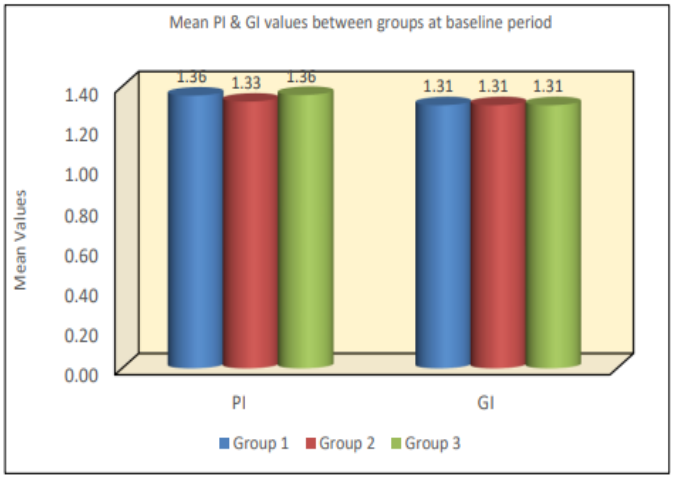
Groups	Time	N	Mean	SD	P-Value [a]	Sig. Diff	P-Value [b]
Group 1	Baseline	14	0.71	0.83	0.10	BL vs 3M	..
	3 Months	14	1.21	1.12		BL vs 6M	..
	6 Months	14	1.36	1.15		3M vs 6M	..
Group 2	Baseline	14	0.57	0.76	0.13	BL vs 3M	..
	3 Months	14	0.43	0.65		BL vs 6M	..
	6 Months	14	0.14	0.36		3M vs 6M	..
Group 3	Baseline	14	0.57	0.85	0.002*	BL vs 3M	0.02*
	3 Months	14	1.21	1.19		BL vs 6M	0.009*
	6 Months	14	1.36	1.28		3M vs 6M	0.49

* - Statistically Significant.

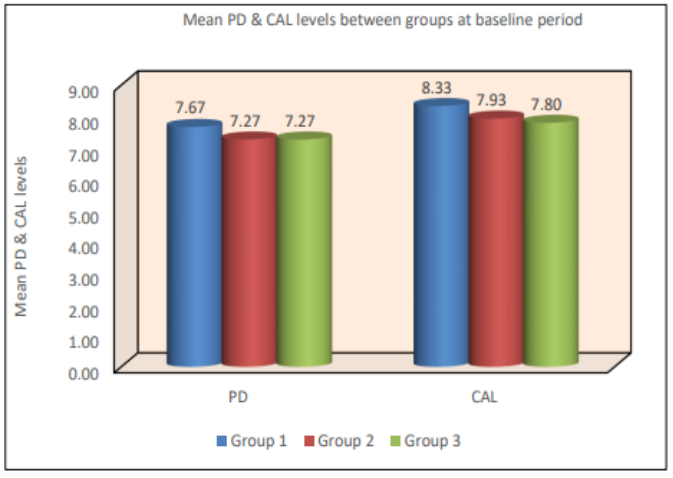
Table 13: Comparison of mean defect depth (in mm) between diff. time intervals in each study group.

Groups	Time	N	Mean	SD	P-Value [a]	Sig. Diff	P-Value [b]
Group 1	Baseline	14	4.46	0.93	<0.001*	BL vs 3M	<0.001*
	3 Months	14	2.79	0.78		BL vs 6M	<0.001*
	6 Months	14	1.89	0.90		3M vs 6M	0.001*
Group 2	Baseline	14	3.75	0.47	<0.001*	BL vs 3M	<0.001*
	3 Months	14	2.39	0.45		BL vs 6M	<0.001*
	6 Months	14	1.32	0.77		3M vs 6M	<0.001*
Group 3	Baseline	14	4.04	0.75	<0.001*	BL vs 3M	<0.001*
	3 Months	14	3.07	0.62		BL vs 6M	<0.001*
	6 Months	14	2.36	0.77		3M vs 6M	<0.001*

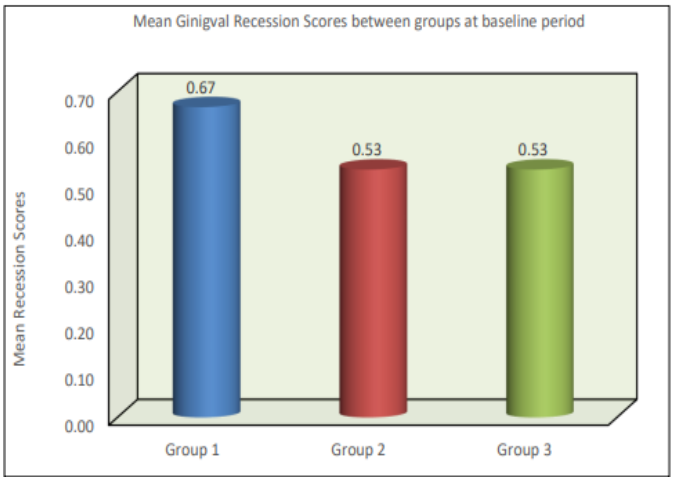
Graph I: Comparison of plaque score and gingival score between the groups at baseline.



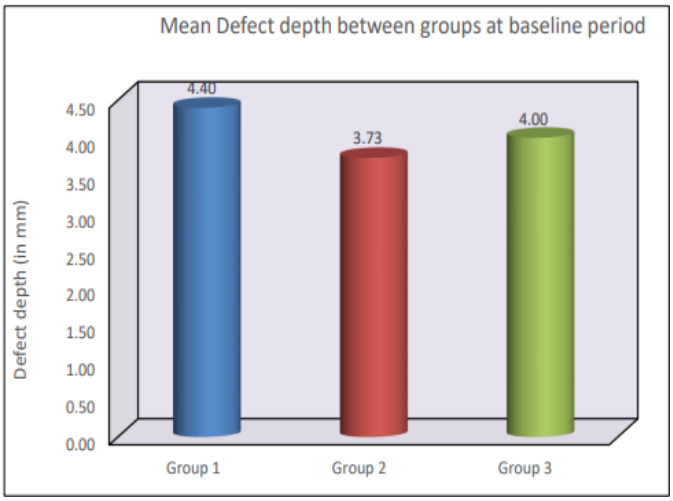
Graph II: comparison of pocket depth (PD) and clinical attachment level (CAL) between the groups at baseline.



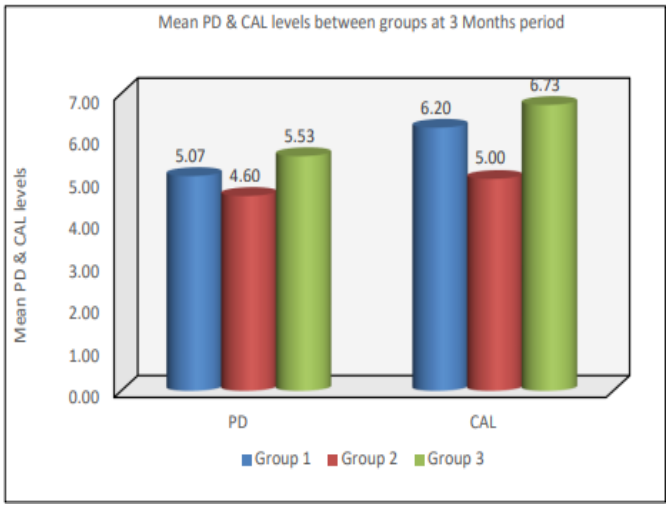
Graph III: comparison of gingival recession (GR) between the groups at baseline.



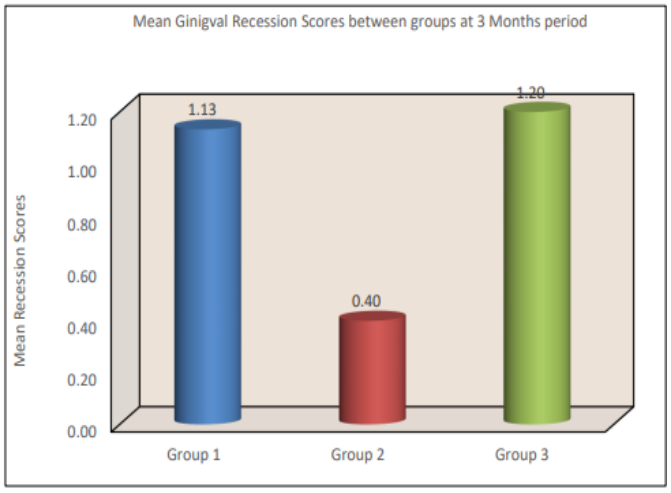
Graph IV: Comparison of the depth of defect (DOD) between the groups at baseline.



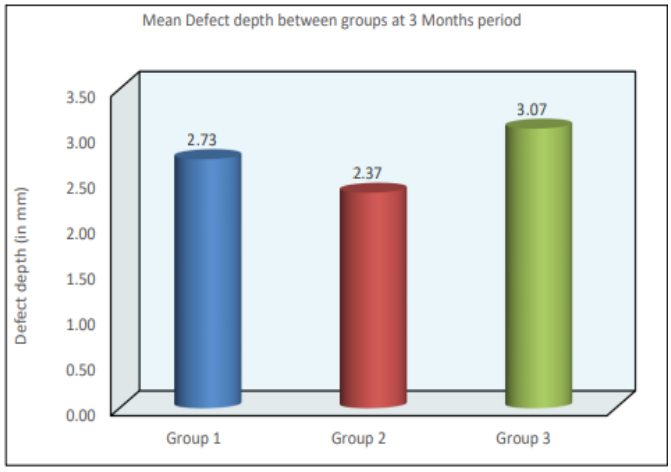
Graph V: comparison of pocket depth (PD) and clinical attachment level (CAL) between the groups at 3 months.



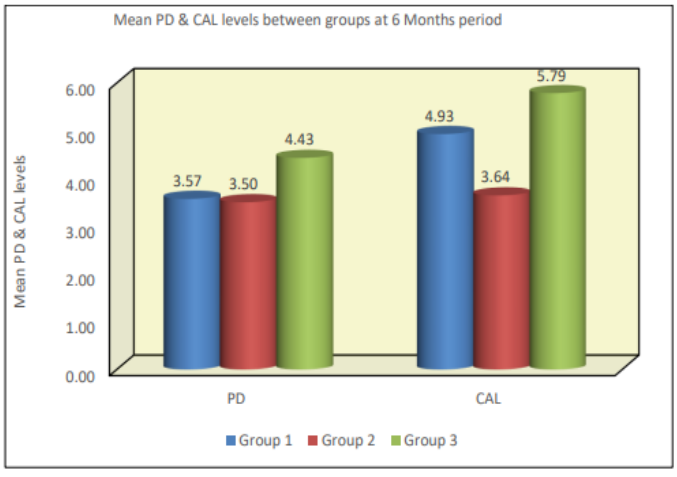
Graph VI: Comparison of gingival recession (GR) between the groups at 3 months.



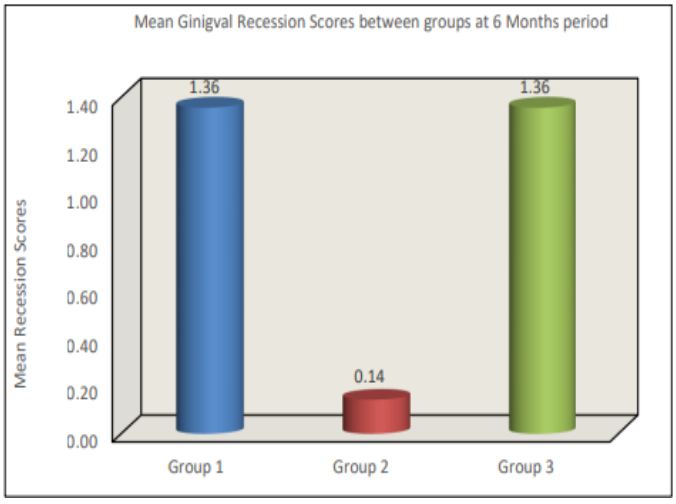
Graph VII: Comparison of the depth of defect (DOD) between the groups at 3 months.



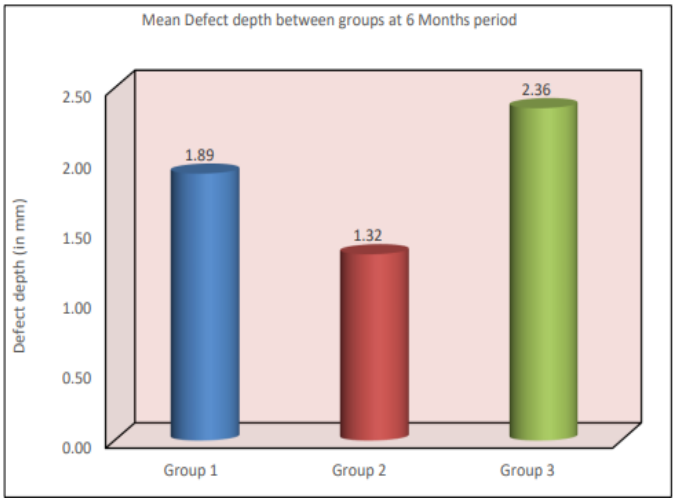
Graph VIII: Comparison of pocket depth (PD) and clinical attachment level (CAL) between the groups at 6 months.



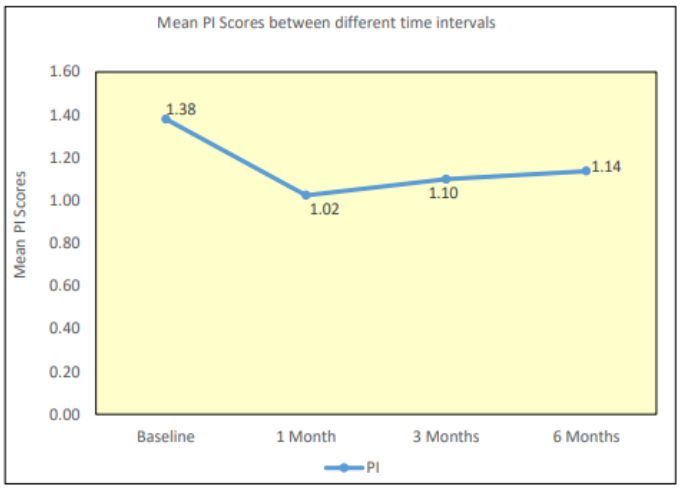
Graph IX: Comparison of gingival recession (GR) between the groups at 6 months.



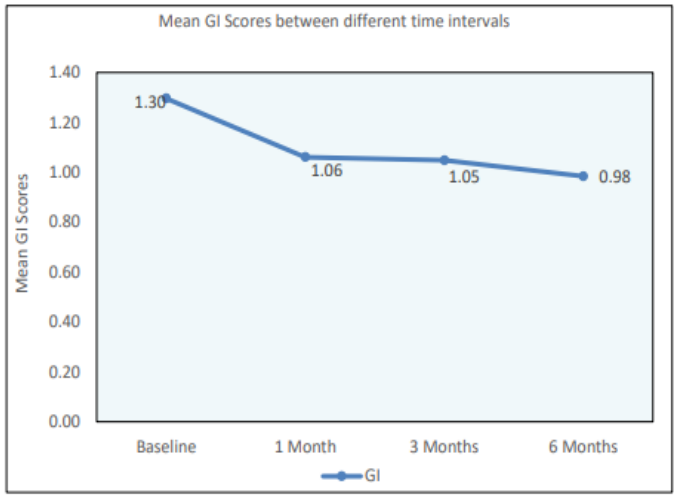
Graph X: Comparison of the depth of defect (DOD) between the groups at 6 months.



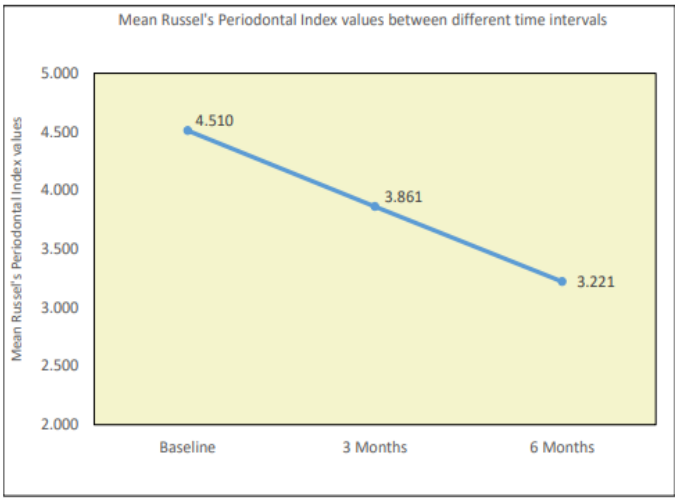
Graph XI: Comparison of mean plaque index scores between the groups at different time intervals.



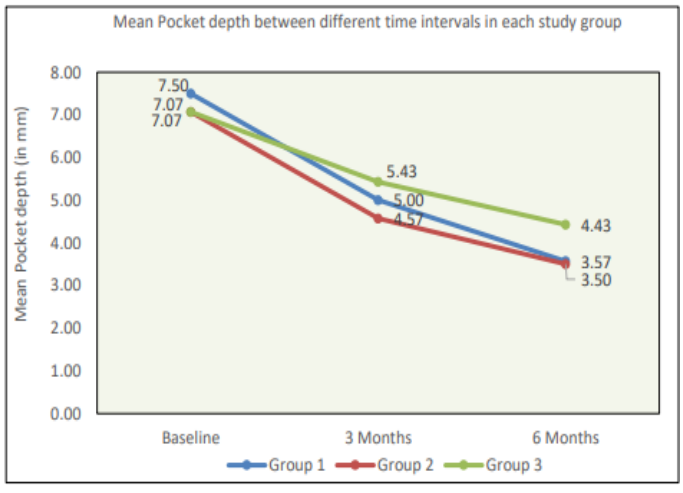
Graph XII: Comparison of mean gingival scores between the groups at different time intervals.



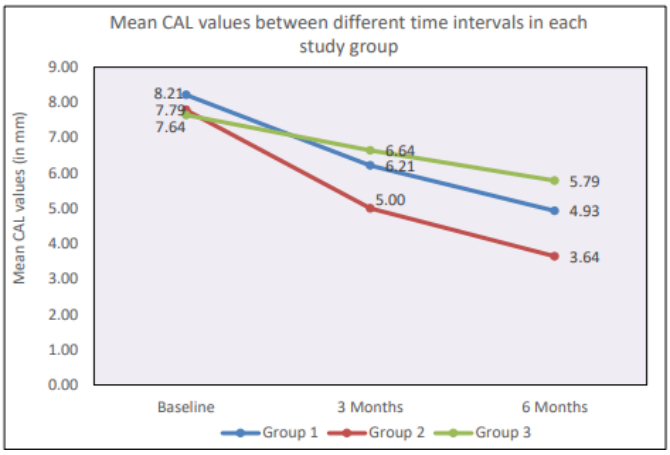
Graph XIII: Comparison of mean Russel's periodontal index between the groups at different time intervals.



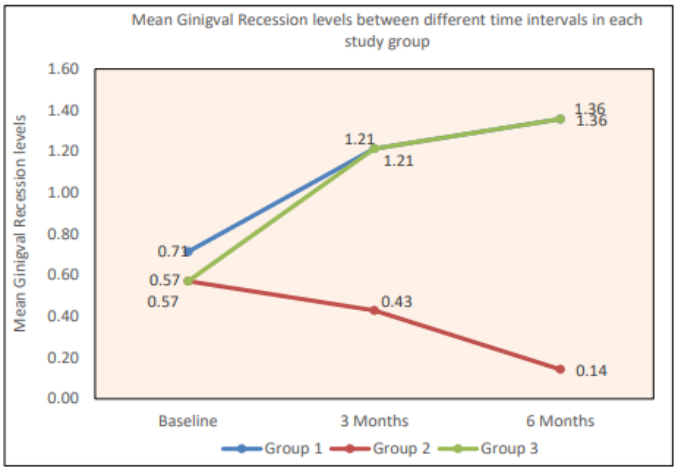
Graph XIV: Comparison of mean pocket depth (PD) between the groups at different time intervals.



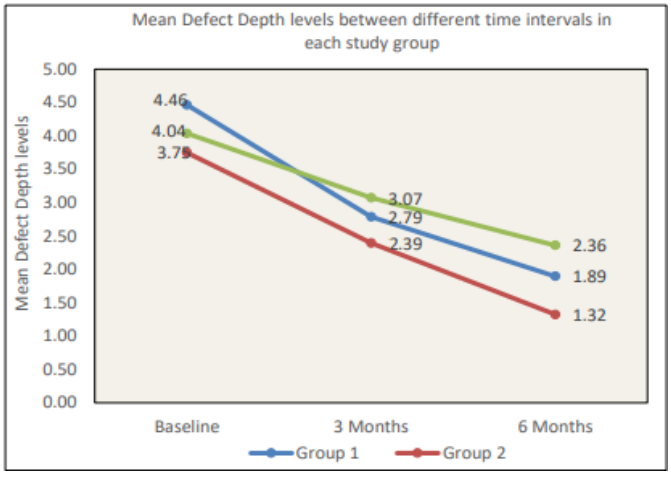
Graph XV: Comparison of mean clinical attachment level (CAL) between the groups at different time intervals.



Graph XVI: comparison of mean gingival recession (GR) between the groups at different time intervals.



Graph XVII: comparison of the mean depth of the defect (DOD) between the groups at different time intervals.



Group I (PRF + 1% METFORMIN).

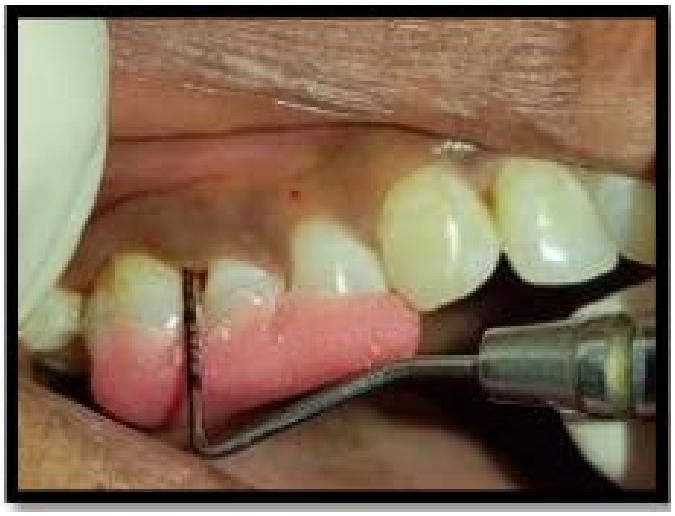


Figure 1A: pre-operative clinical examination.

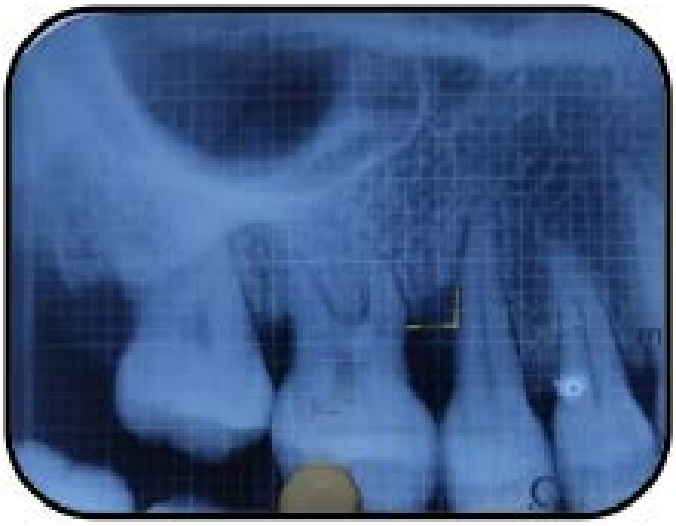


Figure 1B: pre-operative radiographic examination.

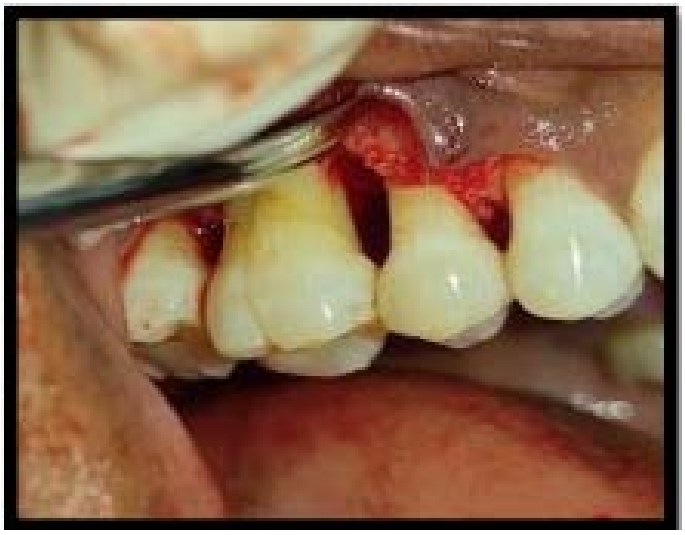


Figure 1C: Intraoperative view of the defect.

Figure 1D: Platelet-rich fibrin + 1% Metformin gel.

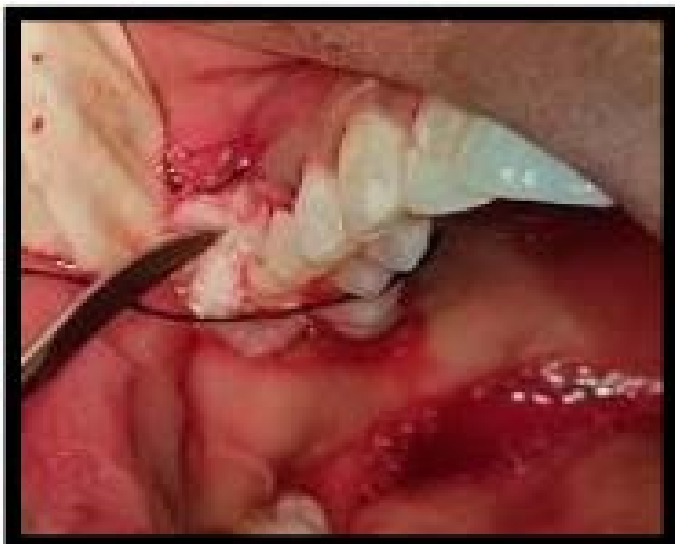


Figure 1E: Placement of 1% Metformin gel.

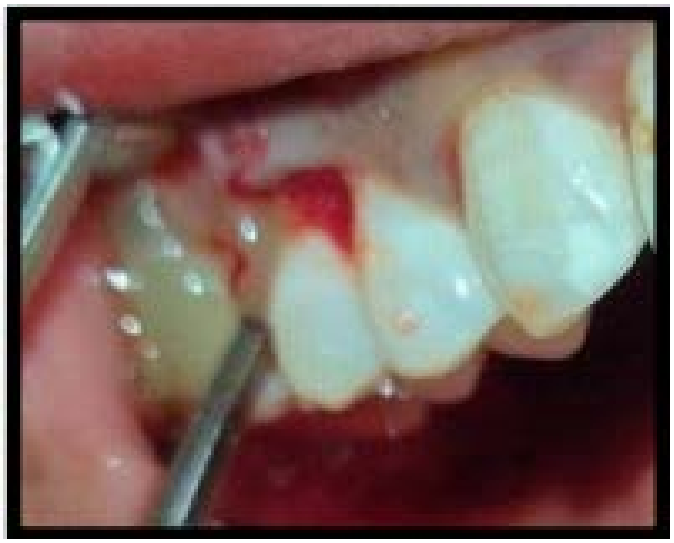


Figure 1F: Placement of Platelet-rich fibrin.

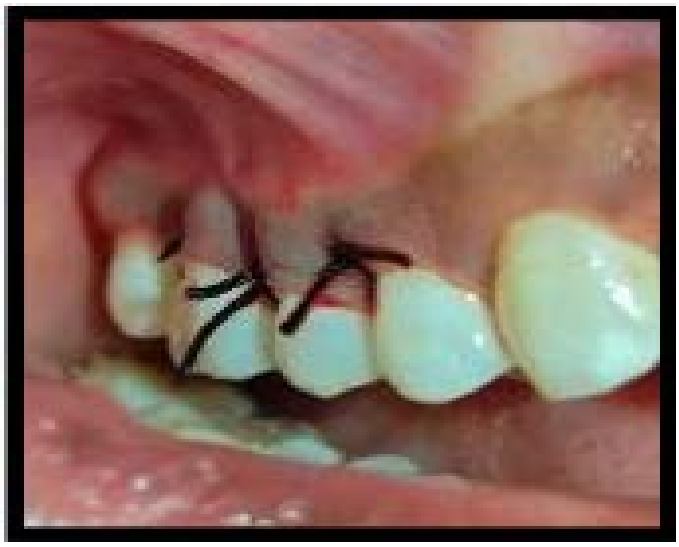


Figure 1G: Sutures placed.

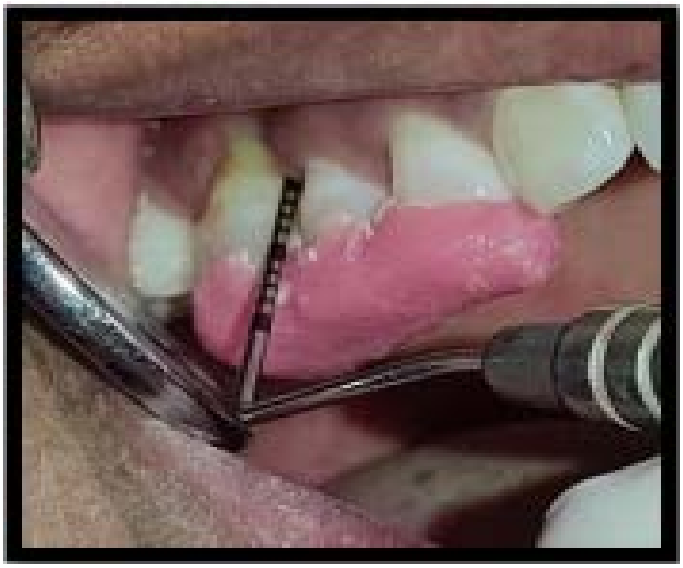


Figure 1H: post-operative clinical evaluation.

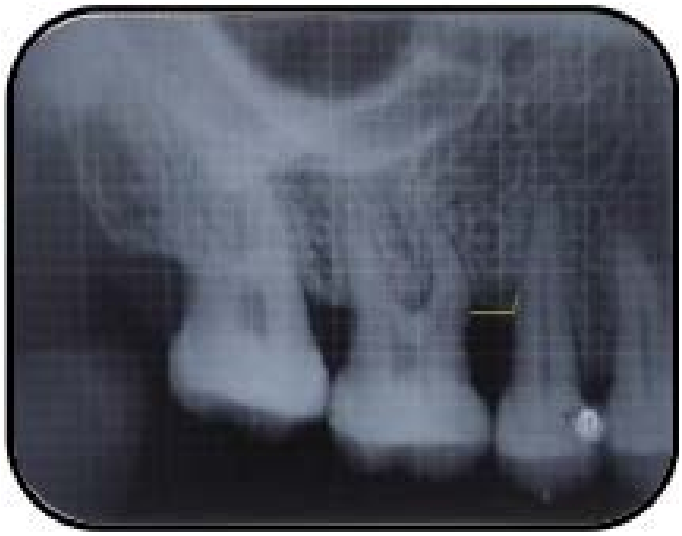


Figure 1I: post-operative radiographic evaluation.

Group II – OFD and PRF.

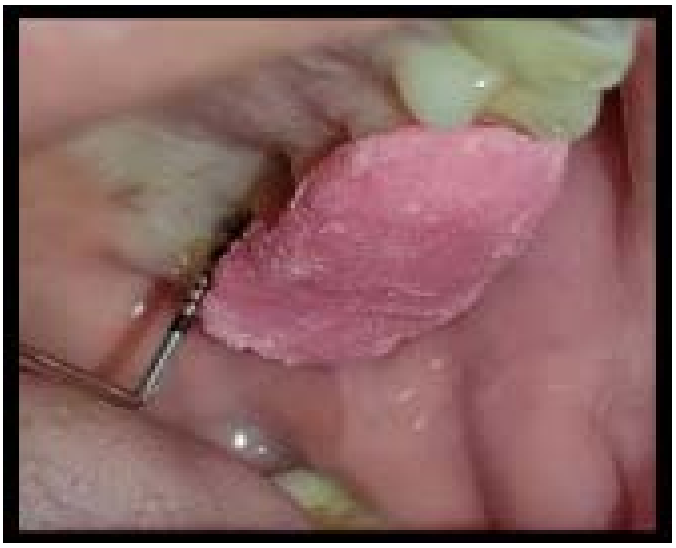


Figure 2A: pre-operative clinical examination.

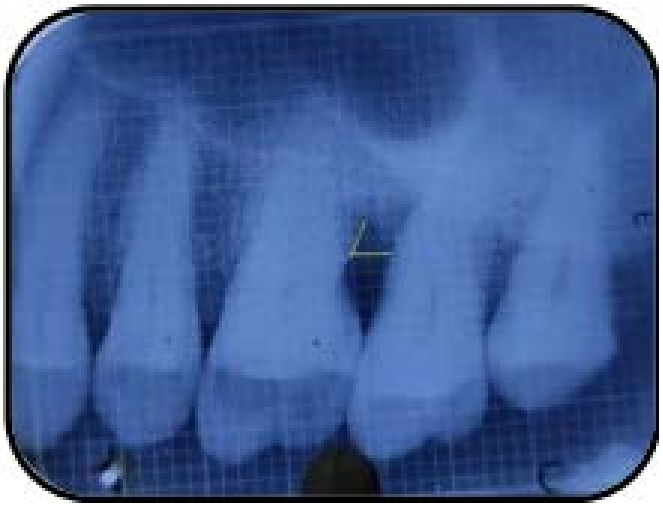


Figure 2B: pre-operative radiographic examination.

Figure 2C: Intraoperative view of the defect.

Figure 2D: Platelet-rich fibrin + 1% Metformin gel.

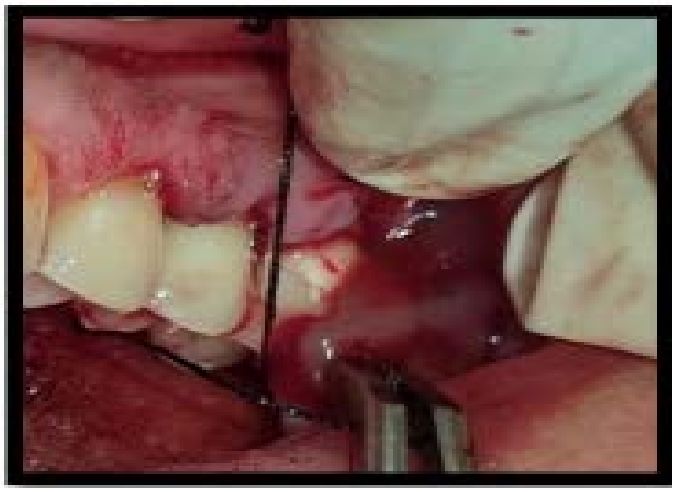


Figure 2E: Placement of Platelet-rich fibrin.

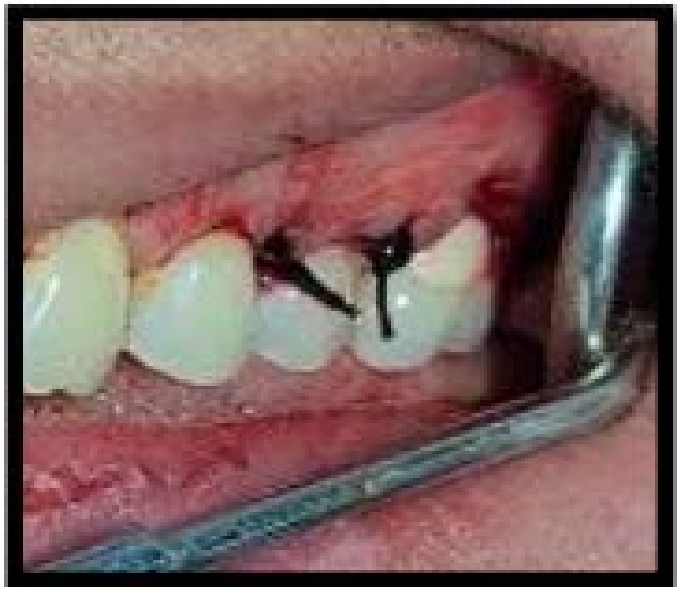


Figure 2F: Sutures placed.

Figure 2G: post-operative clinical evaluation.

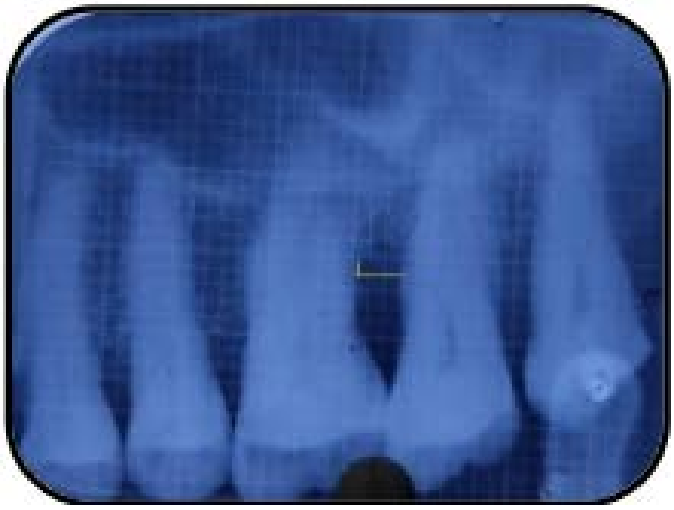


Figure 2H: post-operative radiographic evaluation.

Group iii: OFD alone.

Figure 3A: pre-operative clinical examination.

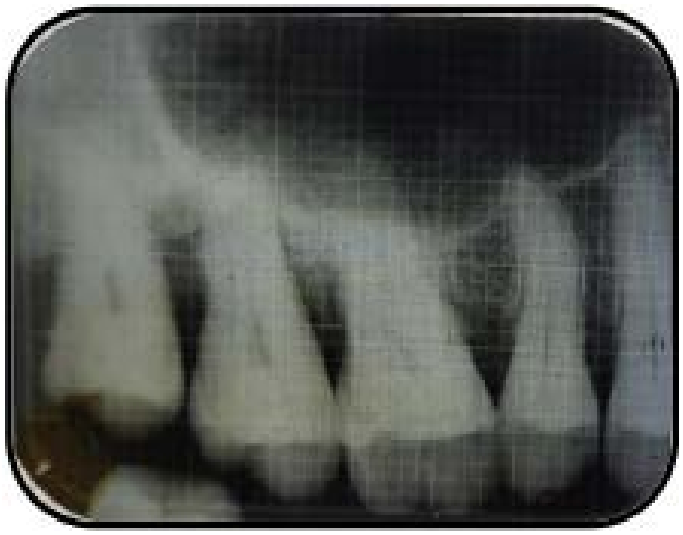


Figure 3B: pre-operative radiographic examination.

Figure 3C: Intraoperative view of the defect.

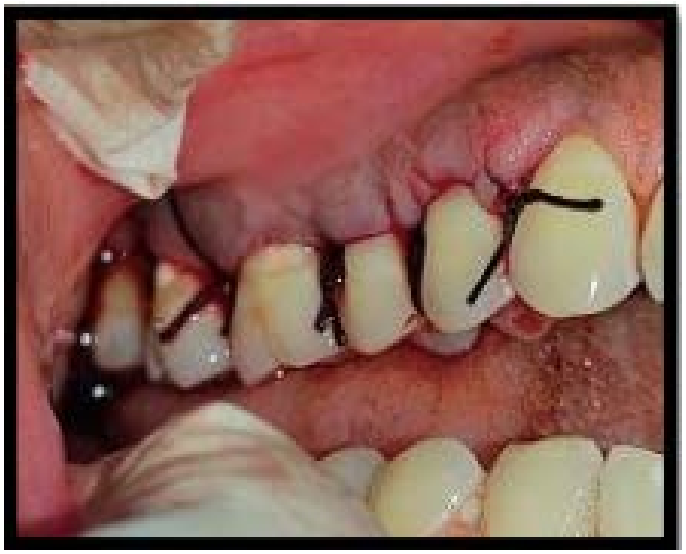


Figure 3D: Sutures placed.

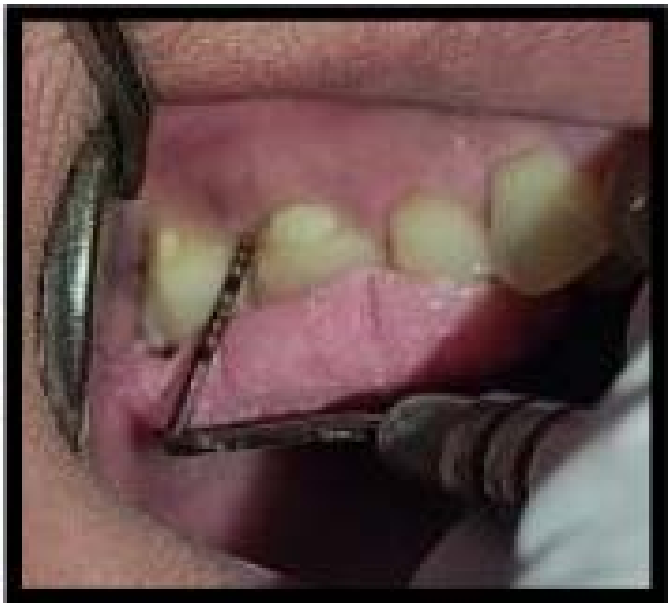


Figure 3E: post-operative clinical evaluation.

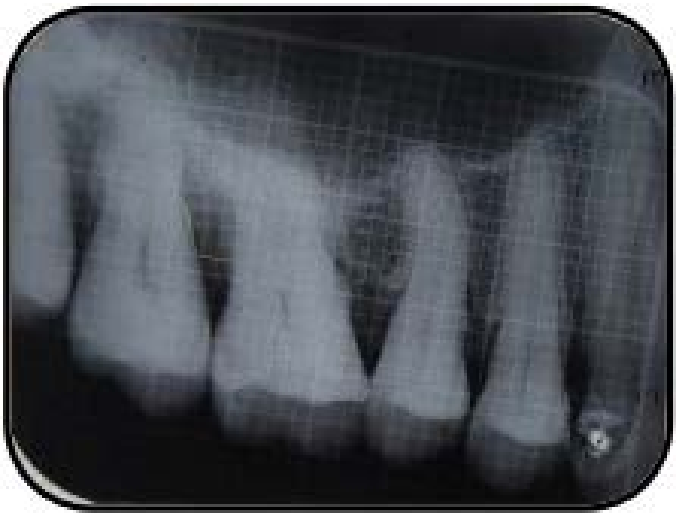


Figure 3F: post-operative radiographic evaluation.