Original Article

Use of Platelet Rich Plasma in the Management of Periodontal Intra-Osseous Defects: A Clinical Study

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ABSTRACT

Background: Periodontal disease is characterized by the presence of gingival inflammation, periodontal pocket formation, loss of connective tissue attachment, and alveolar bone around the affected tooth. Alveolar bone support and attachment apparatus regeneration has been achieved through various processes and have given elusive results. An expedient and cost-effective approach to obtain autologous platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β is the use of platelet-rich plasma (PRP). PRP is obtained by sequestrating and concentrating platelets by gradient density centrifugation.
Aims: The current study was aimed at evaluating the regenerative potential of platelet-rich plasma in comparison with open flap debridement.
Settings and Designs: This study was a randomized controlled clinical trial conducted in the Department of Periodontics and Oral Implantology, KIDS, Bhubaneswar, Odisha.
Materials and Methods: Twenty periodontal infrabony defects in 10 patients; 6 males and 4 females of age between 25–45 years were included in this study and were followed up for a period of 6 months.
Statistical Analysis: Both the groups showed a mean plaque index of 2.10 and 2.50 at baseline, 1.75 and 2.05 at 3 months, and 1.28 and 1.53 at the end of 6 months. The mean reduction of 0.35 and 0.45 at three months and 0.82 and 0.97 at six months was achieved, which was statistically significant. ($P < 0.001$). When comparison was done between the two groups it was not found to be statistically significant ($P < 0.05$). In each of the group there was definitive reduction in plaque score over a period of time.
Results and Conclusion: There was no statistically significant difference in the treatment outcome between open flap debridement and PRP alone. Platelet-rich plasma application holds promise and needs further exploration.

KEYWORDS: *Angular bone defects, periodontal regeneration, periodontitis, platelet-rich plasma PRP*

INTRODUCTION

Periodontal disease is one of the most prevalent afflictions worldwide and is the major cause of tooth morbidity and mortality. The disease is characterized by the presence of gingival inflammation, periodontal pocket formation, loss of connective tissue attachment, and alveolar bone around the affected tooth. Alveolar bone support and attachment apparatus regeneration has been achieved through various processes and has given elusive results.[1] This goal can be accomplished using regenerative surgical procedure such as root biomodification, use of bone replacement grafts, guided tissue regeneration (GTR), and growth factors.[1,2]

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There is evidence to suggest that present regenerative technique lead to significant amounts of regeneration at localized sites on specific teeth. However, if complete regeneration is to become a reality, additional stimuli to enhance the regenerative process are likely needed. Perhaps these could be attempted with polypeptide growth factors or biologic modifiers to provide additional stimulus.[3]

A convenient and economical approach to obtain autologous platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β is the use of platelet-rich plasma (PRP).[4] PRP is obtained by sequestrating and concentrating platelets by gradient density centrifugation. The process of centrifugation concentrates human platelets 338% with identified PDGF and TGF-β within the concentrates.[5,6] Delivery of autologous platelets to periodontal wounds can increase the local concentration of growth factors, which may enhance the healing outcomes.

The aim of current randomized control trial was to evaluate the regenerative potential of platelet-rich plasma.

MATERIALS AND METHODS

This was a prospective randomized controlled clinical trial conducted in the Department of Periodontics and Oral Implantology, KIDS, Bhubaneswar, Odisha. The study protocol was approved by the institutional ethical review board.

The study sample included 20 periodontal infrabony defects in 10 patients; 6 males and 4 females of age between 25–45 years. The defects were randomly divided into two groups with a flip of coin method and followed up for a period of 6 months.

The 20 sites selected for the study were randomly divided into two groups of ten sites each.

Group A: Open flap debridement (Control)
Group B: PRP (Experimental)

INCLUSION CRITERIA

Patients with good general health without any history of systemic disease or compromising medical conditions, clinical evidence of periodontal pocket with probing depths more than 5 mm, having radiographic evidence of intra bony defect, and those willing to co-operate and complete the duration of the study were included.

EXCLUSION CRITERIA

Patients having unacceptable oral hygiene during presurgical phase (phase 1 therapy), with history of antibiotics or other medications affecting the periodontium, within the previous six months, pregnant women and lactating mothers, smokers were excluded from the study.

Comprehensive medical and dental history was recorded and the patients were informed regarding the benefits and protocol of the study and an informed consent was obtained from all the patients.

INITIAL THERAPY (PRESURGICAL THERAPY)

Consisted of oral hygiene instructions, thorough full mouth scaling and root planing. Six weeks following phase-1 therapy, a periodontal re-evaluation was performed to confirm the suitability of the sites for the study. The patients who showed consistently high level of plaque during assessment were not included in the study. Baseline clinical parameters were recorded after revaluation of initial therapy.

CLINICAL PARAMETERS ASSESSED

The following clinical parameters were assessed at baseline, three and six months after the surgical procedure. Radiographic bone levels were assessed at the end of six months and plaque index (PI),[7] gingival index (GI),[8] position of gingival margin (PGM), probing pocket depth (PPD), clinical attachment level (CAL), and radiographic bone level (RBL) were also assessed.

Position of gingival margin, probing pocket depth, and clinical attachment level were recorded by using a UNC-15 probe and a customized acrylic-stent with a guiding groove. This provided well defined and reproducible clinical measurements at each experimental and control site at baseline, three and six months. All customized acrylic stent were stored on the prepared study casts throughout the study period to minimize distortion [Figure 1].

The following measurements were recorded:[9]
1. The distance from reference point (RP) on the stent to the gingival margin (GM)
2. The distance from reference point on the stent to the base of the pocket (BOP)

PPD was recorded by noting the difference between measurements from reference point to gingival margin and reference point to the base of the pocket.

PPD = RP to BOP − RP to GM

Changes in the marginal gingival position were recorded by measuring the distance from fixed reference point to gingival margin.

RADIOGRAPHIC ASSESSMENT

Prior to surgery, intra oral periapical radiographs were taken using an Extension Cone Paralleling Device (Kodak E speed films were used in a Siemens X-ray unit (70 Kv, 15 ma, 0.6 mas) and routine radiographs were also taken.[10] To standardize radiographic assessment, radiographs were obtained in a constant and reproducible plane using film holders with a template, which was

placed in a constant position on a group of teeth and an extension arm that could be attached to the film as well as the X-ray tube. Intraoral periapical (IOPA) radiographs were taken at baseline and at six months, postoperatively.

METHOD FOR RADIOGRAPHIC ASSESSMENT

The following method was employed to determine radiographic changes before and after the study.[11]

1. Preoperative measurement at baseline

A – CEJ to base of defect (BOD)
B – CEJ to the Alveolar crest (AC)
C – Defect depth at base line (A-B)

2. Postoperative measurements at six months

A^1 – CEJ to BOD
B^1 – CEJ to AC
C^1 – Defect depth at six months $(A^1 \pm B^1)$
E – Changes in alveolar crest at six months $(B \pm B^1)$
D – Defect fill in mm (linear). $(C \pm C^1)$

ARITHMETIC DETERMINATIONS

1. Defect depth in mm. $C = A \pm B$ $C^1 = A^1 \pm B^1$
2. Defect fill in mm. $D = C \pm C^1$ $D^1 = C \pm C^2$
3. Changes in alveolar crest. $E = B \pm B^1$ $E1 = B \pm B^2$
4. Percentage of defect fill = [Defect depth at base line – Defect depth (six months)] – Change in alveolar crest (six months) / Defect depth at baseline ×100
5. Percentage of original defect resolution = Defect depth at base line - Defect depth (six months) / Defect depth at baseline × 100

The radiographs were scanned on an HP X-ray scanner and measurements were carried out by the help of image analysis software (Auto-Cad) to see changes between preoperative and postoperative radiographs.

PREPARATION OF PLATELET-RICH PLASMA

PRP was prepared according to the procedure described by Kazuhiro Okuda *et al.*[12] One hour prior to the periodontal surgery 8–10 ml of whole blood was drawn from the patient's antecubital vein. Blood was collected in a vaccutainer coated with an EDTA. The tubes were inverted several times to ensure the mixing of blood and anticoagulant. The sample tube is then spun in a standard centrifuge for 10 minutes at 2400 rpm to separate PRP and platelet-poor plasma (PPP) from the red blood cell fraction. Leaving just 1 ml of PPP above the buffy coat, rest was discarded. This preparation (1 ml of PPP + buffy coat + 1 ml of red blood cell fraction rich in newly synthesized platelets) was pippetted out into a test tube without an anticoagulant. Separation of PRP and PPP was done by centrifugation at 3600 rpm for 15 minutes and was drawn into a sterile syringe [Figures 2-4].

Occlusal stent using pink polymerizing resin were then fabricated in the area of interest for standardization [Figure 5].

PRESURGICAL CLINICAL MEASUREMENTS

The PI, GI, PGM, PPD, and CAL were recorded.

SURGICAL PROCEDURE

Intracrevicular incisions were made and mucoperiosteal flap were elevated, to preserve as much as soft tissue in order to obtain primary closure. Using said periodontal surgical procedure, the intrabony defects were fully exposed and thorough granulation tissue debridement and root planing were carried out to remove subgingival plaque, calculus, and pocket epithelium from the defect with continuous saline irrigation [Figures 6 and 7]. Interdental direct suturing technique was used to approximate the flaps [Figure 8]. Immediately before application the PRP was activated by clot initiator, 10% calcium chloride, and whole blood from the defect, within a few seconds the PRP preparation assumed a sticky gel consistency that was relatively easy to apply to the surgical defects. The coagulated PRP was placed up to the vertical height of the corresponding adjacent bone level. Flaps were repositioned to the pre-surgical level and the previously placed loose sutures at the sites were approximated and stabilised to achieve a primary closure and periodontal dressing was placed on the surgical area.

POSTTREATMENT ASSESSMENTS

PI, GI, PGM, PPD, and CAL were recorded at three and six months and radiographic assessments were done after six months postoperatively and oral hygiene instructions were reinforced [Figure 9-13].

STATISTICAL ANALYSIS

The data at baseline, three and 6 months after surgery was first collected. Following this, the data was subjected to statistical analysis using analysis of variance (ANOVA) and paired *t*-test.

All data were expressed as mean (SD). Statistical analysis was performed using a commercial SPSS version II. One-way analysis of variance was applied to examine the difference among the four groups. Appropriate levels of significance were obtained with the *t* and *P* values, when the clinical and radiographic parameters were subjected to student *t*-test.

RESULTS

PLAQUE INDEX

Group A and Group B showed a mean plaque index of 2.10 and 2.50 at baseline, 1.75 and 2.05 at three months, and 1.28 and 1.53 at the end of six months. The mean

reduction of 0.35 and 0.45 at three months and 0.82 and 0.97 at six months was achieved, which was statistically significant. (P <0.001) [Figure 14].

COMPARISONS BETWEEN THE GROUPS

When comparison was done between the two groups it was not found to be statistically significant (P <0.05). In each of the group there was definitive reduction in plaque score over a period of time.

GINGIVAL INDEX

Comparisons between the groups

Baseline gingival score

The mean gingival index at baseline for Group A was 2.45 ± 0.48 and for Group B was 2.33 ± 0.65. No statistical significance was found [Figure 15].

Gingival score at the end of three months

The mean gingival index for Group A at three months was 2.18 ± 0.47 and for Group B was 2.05 ± 0.67. It was not found to be statistically significant [Figure 15].

Gingival score at the end of six months

The mean gingival index at six months for Group A was 1.80 ± 0.35 and for Group B was 1.35 ± 0.58, and it was statistically significant. When comparison was done between the two study groups it was not found to be statistically significant (P <0.05), but each group showed improvement in gingival condition over a period of time [Figure 15].

POSITION OF GINGIVAL MARGIN

Comparisons between the groups

Baseline values

The mean PGM at baseline for Group A was 2.55 ± 0.37 and for Group B was 2.40 ± 0.57. It was not found to be statistically significant [Figure 16].

Values at the end of three months

The mean PGM for Group A at three months was 2.28 ± 0.34 and for Group B was 2.13 ± 0.49. It was not found to be statistically significant [Figure 16].

Values at the end of six months

The mean PGM at six months for Group A was 2.08 ± 0.37 and for Group B was 1.94 ± 0.47. It was not found to be statistically significant result [Figure 16].

When comparison was done between the two study groups it was not found to be statistically significant (P < 0.05). In all the groups, the procedures showed some degree of gingival shrinkage at the end of study period.

PROBING POCKET DEPTH

Comparisons between the groups

Baseline values

The PPD values for the sites ranged from 6 to 10 mm with a mean of 7.3 ± 0.94 mm for Group A and 8 ± 1.24 mm for Group B. It was not found to be statistically significant [Figure 17].

Values at the end of three months

At three months, PPD values for the two groups ranged from 3 mm to 6 mm with a mean of 5.2 ± 0.79 mm for Group A and 5.3 ± 1.16 mm for Group B [Figure 17].

Values at the end of six months

At six months, PPD values for the two groups ranged fro 2 mm to 5mm with a mean of 3.4 ± 0.88 mm for Group A, 3.70 ± 1.06 mm for Group B. It was not found to be statistically significant [Figure 17].

When comparison was done between the two groups, it was not found to be statistically significant (P < 0.05). All the groups resulted in significant reductions in probing pocket depth [Figure 17].

CLINICAL ATTACHMENT LEVEL

Comparisons between the groups

Baseline values

The CAL values for the sites ranged from 8 to 13 mm with a mean of 11.70 ± 1.64 mm for Group A and 12.70 ± 1.49mm for Group B. It was found to be statistically significant [Figure 18].

Values at the end of three months

At three months, clinical attachment level values for the two groups ranged from 7mm to 11 mm with a mean of 10.00 ± 1.65 mm for Group A , 10.80 ± 1.39 mm for Group B. It is found to be statistically significant.

Values at the end of six months

At six months, clinical attachment level values for the two groups ranged from 8mm to 11 mm with a mean of 8.20 ± 1.52 mm for Group A, 9.00 ± 1.45 mm for Group B. It is found to be statistically significant [Figure 18].

When comparison was done between the two groups it was found that both the groups resulted in significant reductions in CAL

RADIOGRAPHIC BONE LEVEL

The radiographic defect depth values at baseline: Group A showed a mean defect depth of 3.10 ± 1.10 mm and Group B showed 2.90 ± 1.45 mm. The radiographic defect depth values at six months: Group A showed a mean defect depth of 2.30 ± 0.97 mm and Group B showed 2.10 ± 1.25 mm [Figures 19-21].

The radiographic defect fill values: Group A showed a mean defect fill of 0.80 ± 1.03 mm and Group B showed 0.80 ± 0.79 mm [Figure 22].

The percentage of radiographic defect fill in Group A is 27.30 ± 0.30 % and in Group B is 38.40 ± 0.33 % [Figure 23].

The percentage of radiographic resolution of the defect in Group A is 35.90 ± 0.35 % and in Group B is 41.70 ± 0.32% [Figures 24 and 25].

DISCUSSION

The ultimate goal of periodontal therapy is the creation of an environment that is conducive to maintain patient dentition in a state of optimum health, comfort, and function. Regenerative periodontal therapy aims to reform and reconstitute the supporting tissues of teeth, which have been lost due to periodontal disease and trauma. Several regenerative therapeutic procedures have been developed for this purpose; and have met with partial or marginal success. These include root surface biomodification, use of various types of bone grafts, GTR, and combination of the above.

Platelet-rich plasma is an autologous volume of plasma with 4–5 fold increase in platelet concentration, and is a proven source of growth factors such as PDGF, TGF, IGF, VEGF, EGF, platelet-derived angiogenesis factor, and platelet factor IV.[10,12-14] The positive impact of PRP on bone healing is attributed to angiogenic, proliferate and differentiating effect of PDGF and TGF present in high concentration. However, there is pausity of information about the clinical efficacy of PRP in repair and regeneration of periodontal defect. Ten patients (6 males and 4 females) in the age range of 25–45 years

with 20 intrabony defects were enrolled for this study. The defects were randomly grouped into two groups as control (Group A) and experimental (Group B) groups. All the patients completed the study period. No significant complication was observed in any of the treated groups. The parameters and variables assessed were PI, GI, PGM, PPD, CAL in three and six months and radiological assessment was done only at the end of six months.

The plaque and gingival index were assessed at baseline, three months and six months in order to monitor patient's oral hygiene and its effects on the soft tissue as this goes a long way in achieving the desired objective. The results of our investigation showed a statistically significant decrease in the plaque index from baseline to three months and at the end of six months in control (Group A) as well as experimental group (Group B), which is in accordance with the study by Dori *et al*.[13,10] However, no statistically significant difference was recorded between both the groups suggesting that there was maintenance
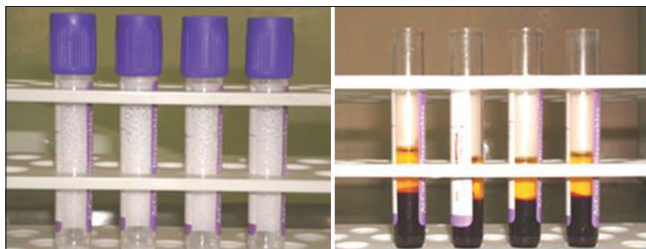

Figure 1: Armamentarium

Figure 3: 2ml vaccutainer and after 1st centrifuge
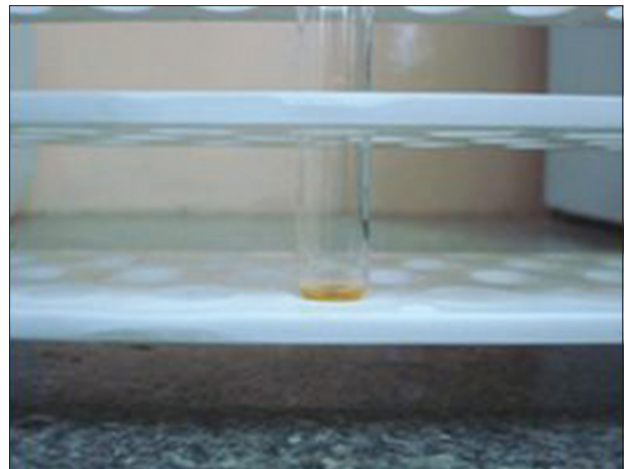

Figure 2: Centrifugal machine

Figure 4: 0.5ml platelet rich plasma

Figure 5: Probing with occlusal stent

Figure 6: Incision placed

Figure 7: Reflection and debridement

Figure 8: Suture placement

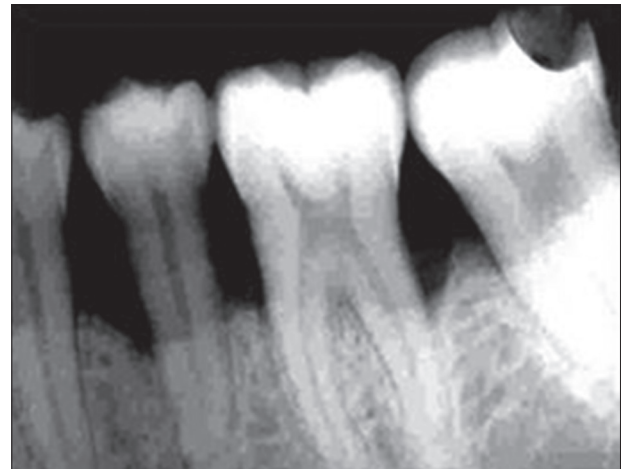Figure 9: Post operative probing depth with occlusal stent

Figure 10: OFD pre operative distal aspect of 36

of good oral hygiene throughout the study in all groups and all patients were very well motivated as oral hygiene plays an important role in determining the treatment outcome. The maintenance of good oral hygiene and

significant reduction in plaque index throughout the study period reflected in gingival health. The result showed a significant reduction in gingival index from baseline to the end of third month and sixth month in both control and
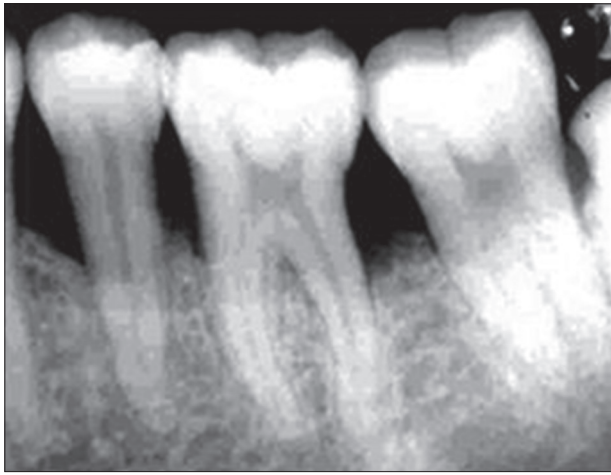
Figure 11: OFD group post operative 6 month distal aspect of 36
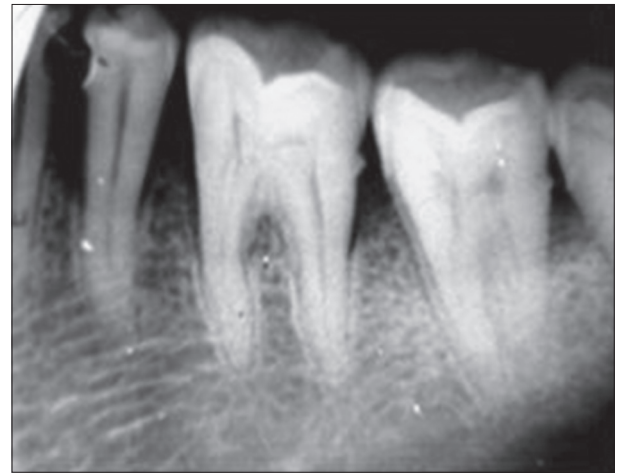
Figure 12: PRP group pre operative distal aspect of 36
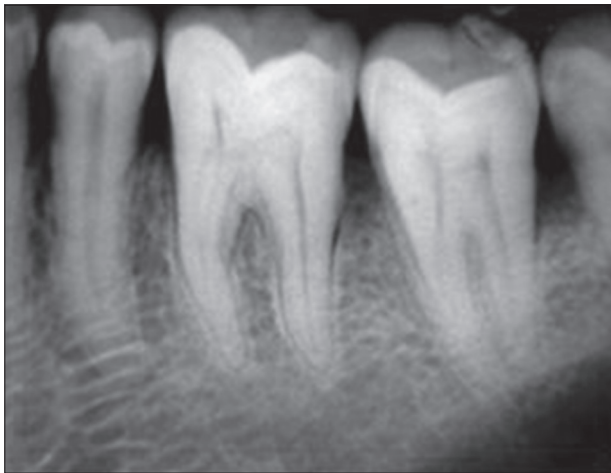

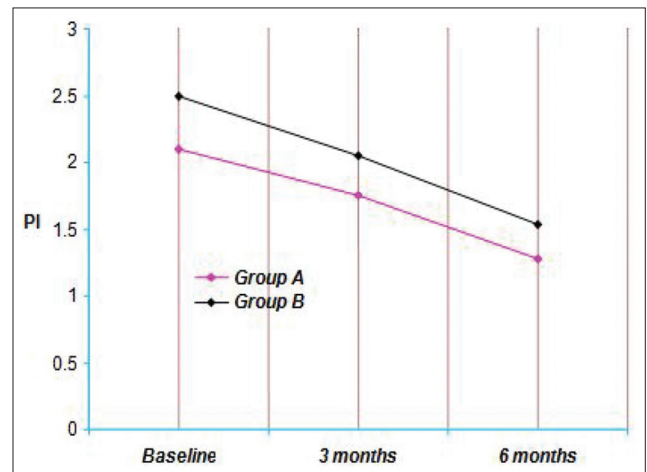Figure 13: PRP group post operative distal aspect of 36

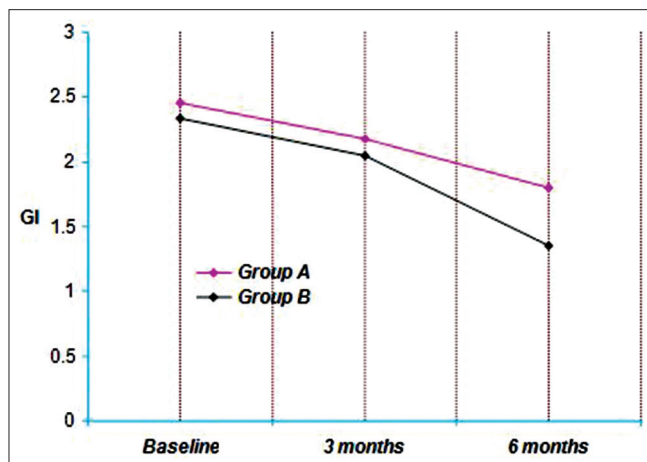Figure 14: The mean plaque index among the study groups at various time intervals

Figure 15: The mean gingival index among the study groups at various time intervals
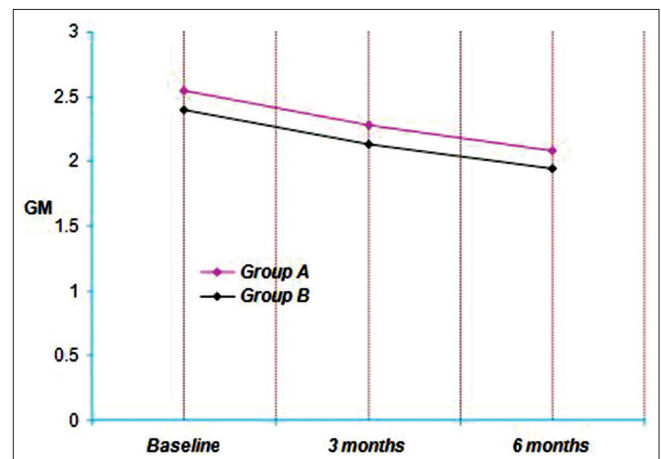

Figure 16: The mean position of gingival margin among the study groups at various time intervals

experimental groups. However, as in the case of plaque index no statistically significant difference was noticed between both the groups suggesting that there was no untoward soft tissue reactions and inflammation in control and experimental sites throughout the study period.

The gold standard for evaluating regeneration is histologic assessment. But this is often not done in clinical trials due to ethical consideration. Surgical re-entry, which is considered next best, also cannot be performed due to morbidity and ethical reasons. Hence, surrogate treatment
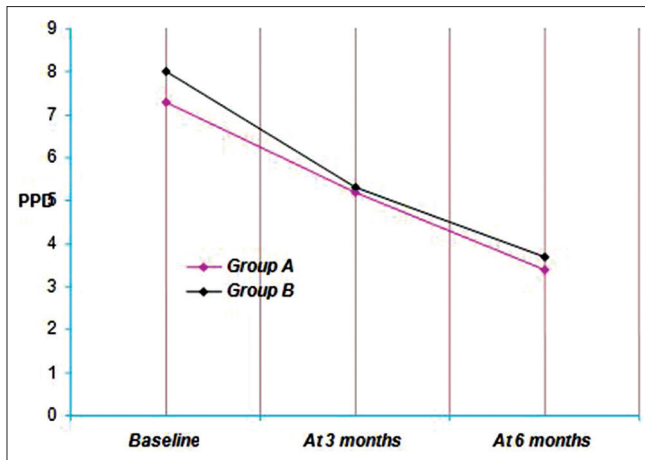
Figure 17: The mean probing pocket depth among the study groups at various time intervals
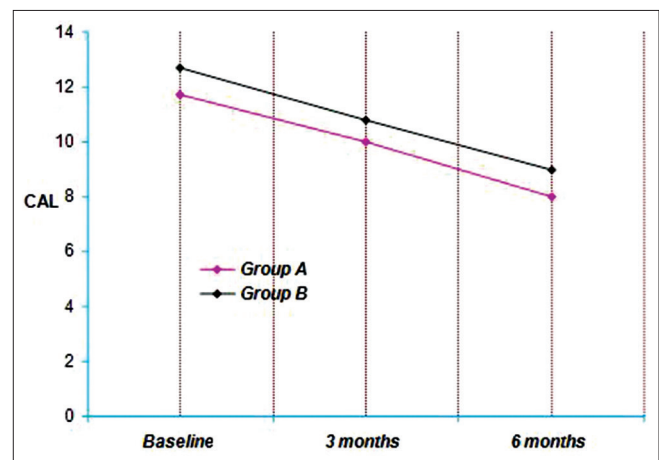

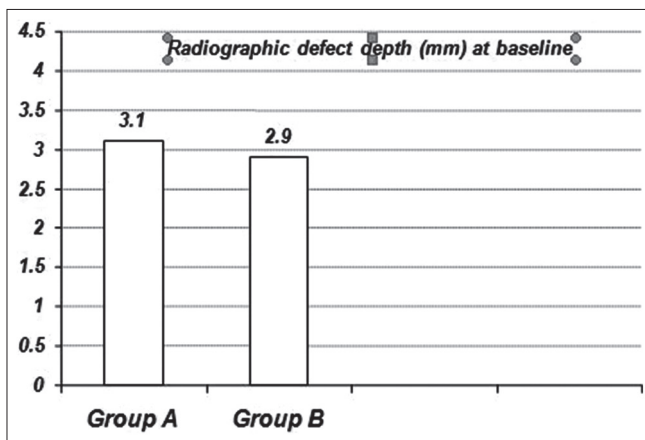**Figure 18:** The mean clinical attachment level among the study groups at various time intervals

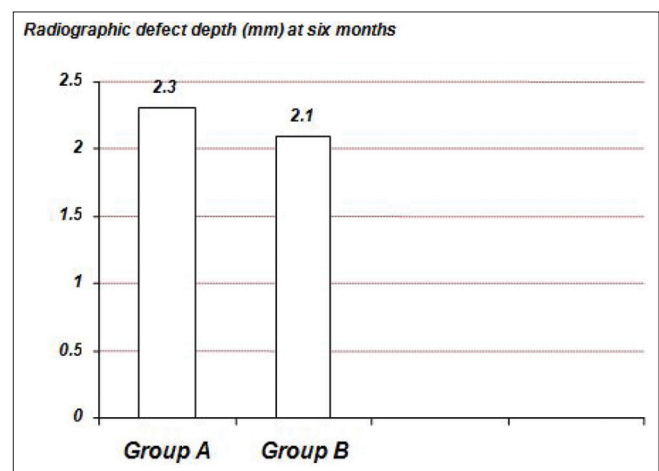**Figure 19:** The mean radiographic defect depth among the study groups at base line

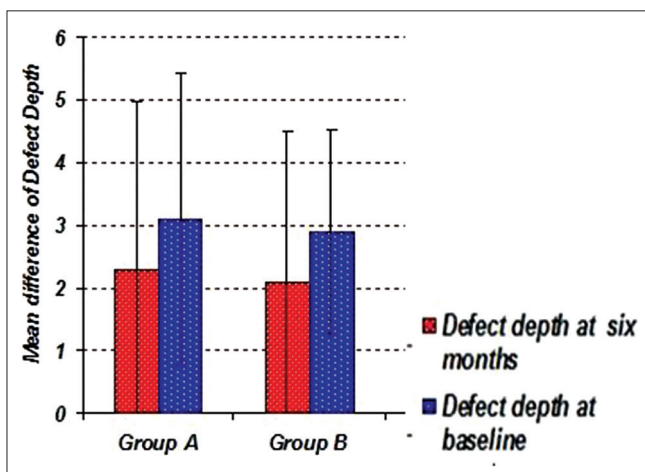**Figure 20:** The mean radiographic defect depth among the study groups at six months

Figure 21: The mean radiographic difference in defect depth among the study groups
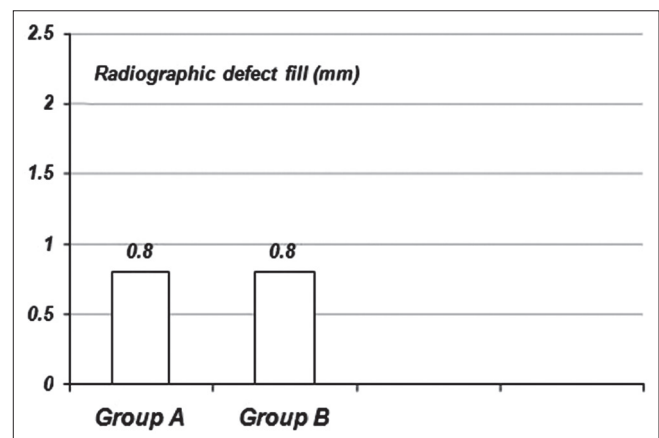

Figure 22: The mean radiographic defect fill at 6 months among the study groups.

outcome measures such as PPD, CAL, and radiographic bone level are often used to indirectly assess the healing and regeneration of periodontal lesions in clinical trials. These measures do not provide the direct proof for new bone formation, new attachment, and regeneration.

The changes in PPD reflect the cumulative effect of the response of gingival tissue to the treatment by way of gingival recession and clinical attachment gain. PPD indicates the volume of subgingival area, which harbors the pathogenic microbiota and favors disease activity.
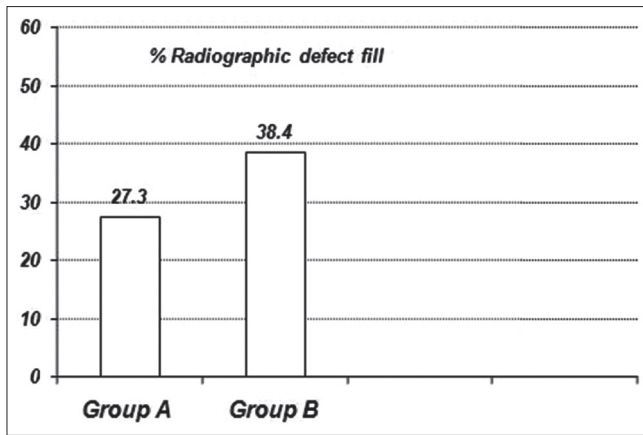
Figure 23: The mean percentage of radiographic defect fill among the study groups at six months
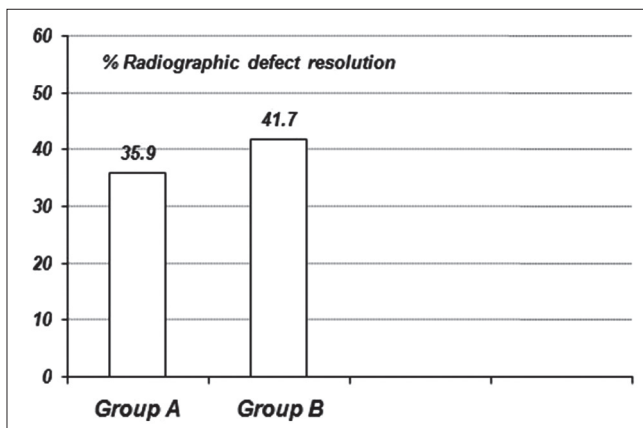

Figure 24: The mean percentage of radiographic defect resolution among the study groups at six months
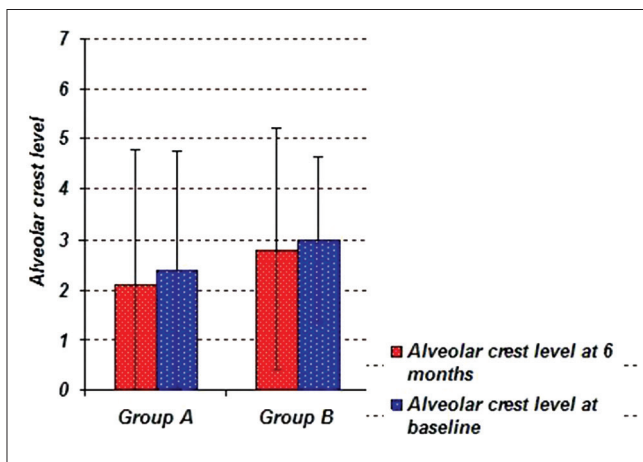

Figure 25: The mean changes in the alveolar crest level at baseline and at six months interval

Hence, it becomes an important variable to be measured for the long term maintenance of periodontal health. The result of the present investigation showed a mean probing depth of 7.30 ± 0.94 mm at baseline, which got reduced significantly to 5.20 ± 0.79 mm at the end of three months and 3.40 ± 0.88 mm at the end of six months in Group A

(control) indicating that there is progressive reduction in PPD at sites treated with open flap debridement (OFD). These findings confirm earlier reports by Heitz- Mayfield LJA *et al.*[15] that OFD results in reduction of periodontal pocket depth. Group B, which was treated with PRP alone, also showed statistically significant reduction in PPD from baseline to three and six months' time interval (8.00 ± 1.24 at baseline, 5.30 ± 1.16 at three months, 3.70 ± 1.06 at six months). However, when compared with Group A, there is no statistically significant change in PPD between the groups suggesting that application of PRP has no added benefit over traditional OFD in reducing PPD. So far a clinically reliable treatment outcome measure for regenerative periodontal therapy apparently has not been established. Change in CAL following regenerative therapy is the single most commonly used outcome measure in regenerative therapy. This is based on reported correlation between gain in CAL and gain in bone height by various clinical studies.[16] The results of our study showed a mean CAL gain of 1.70 mm at three months and 3.50 mm at six months compared to baseline, which was statistically significant. This is in accordance with the study by Heitz- Mayfield *et al.*[17] In group B (1.90 mm at three months and 3.70 mm at the end of six months), the CAL gain was also statistically significant at the end of three and six months period. However, group A when compared with group B showed no statistical significance suggesting that PRP is not superior to open flap debridement. Continuous radiographic evaluation of alveolar bone changes following regenerative procedure is a non-surgical painless alternative to direct bone measurement, which is done by re-entry procedures. Radiographic variables assessed in our study were extent of defect fill and defect resolution using image analysis software (Autocad analysis). As the radiographic changes cannot be appreciated at three months interval they were recorded at six months interval. Our results showed that the mean defect fill for Group A was 0.80 ± 1.3 mm, which is in accordance with the study by Yukna *et al.*[18-20] However, when compared with Group B (0.80 ± 0.79 mm) there was no statistical difference suggesting that the use of PRP alone is not superior to OFD relating to defect fill. This might be because the presence of appropriate cell types, matrix, and signaling molecules are the key to regeneration of tissues. The periodontal ligament cells are reported to be critical in periodontal wound healing and currently periodontal regeneration is based on the concept of promoting repopulation of the wound and the adjacent root surface with cells of periodontal ligament. Periodontal wound healing is a spacio-temporal phenomenon where not only the presence of cell matrix and signaling molecules matters but also the appropriate tissue for the interaction of these elements. The cells of periodontal ligament contain stable cells, which normally

remain quiescent in tissues in G0 and G1 phase waiting for appropriate phase to divide.[21-23] The polypeptide growth factors are reported to have a short half-life and their action is dose dependent. Some of the factors such as PDGF may have an inhibitory effect in cell proliferation at higher concentration.[24-26] The failure in our study for PRP to show superior regeneration compared to OFD may be because of conducive environment for the above described complicated time dependent periodontal wound healing phenomena.[24,27-30] Comparing percentage of defect fill between Group A and Group B - it was not found to be statistically significant.

LIMITATIONS OF THE STUDY

Our study used surrogate outcome measures, which are not a very reliable measure of periodontal regeneration. The intrabony defects included in our study were not identical in width and depth as the dimension of the defect influence the treatment outcome. The study period has been restricted to six months, which is not very ideal for assessing periodontal regeneration either radiographically or clinically. Within the limits of present investigation, it could be concluded that platelet-rich plasma has no added benefit in periodontal regeneration compared to open flap debridement.

CONCLUSION

There was no statistically significant difference in the treatment outcome between OFD and PRP alone. It is yet not clearly known whether PRP alone or with combination with bonegraft would significantly enhance the outcome of regenerative periodontal therapy. PRP application holds promise and needs further exploration.

FINANCIAL SUPPORT AND SPONSORSHIP

CONFLICTS OF INTEREST
There are no conflicts of interest.

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