EFFICACY OF DEMINERALIZED FREEZE-DRIED BONE ALLOGRAFT ALONE AND DFDBA WITH PLATELET RICH FIBRIN IN THE TREATMENT OF INTRABONY DEFECTS: A CLINICAL STUDY

Dissertation

Submitted to

BABU BANARASI DAS UNIVERSITY, LUCKNOW, UTTAR PRADESH

In the partial fulfilment of the requirements for the Degree

Of

MASTER OF DENTAL SURGERY

In

PERIODONTICS

By

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Under the guidance of

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BATCH: 2014-2017

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled "**EFFICACY OF DEMINERALIZED FREEZE-DRIED BONE ALLOGRAFT ALONE AND DFDBA WITH PLATELET RICH FIBRIN IN THE TREATMENT OF INTRABONY DEFECTS: A CLINICAL STUDY**" is a bonafide and genuine research work carried out by me under the guidance of **Dr. Mona Sharma**, Reader, Department of Periodontics, Babu Banarasi Das College Of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

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CERTIFICATE BY THE GUIDE

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I hereby declare that the **Babu Banarasi Das University** shall have the right to preserve, use and disseminate this dissertation in print or electronic format for academic / research purpose.

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"Research can be undertaken in any kind of environment, as long as you have the interest. I believe that true education means fostering the ability to be interested in something."

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Dr. Indu Verma

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ABBREVIATIONS

CEJ	Cemento-enamel junction
FRP	Fixed reference point
BD	Base of the defect
DD	Defect depth
RDA	Radiographic defect angle
D	Diameter of the cone
V	volume of cone
CP	Chronic periodontitis
PI	Plaque index
GI	Gingival index
CAL	Clinical attachment level
PPD	Probing pocket depths
DFDBA	Demineralized freeze-dried Bone Allograft
PRF	Platelet Rich Fibrin

ABSTRACT

Various clinical studies have been carried out to demonstrate that using Demineralized freeze-dried bone allograft and Platelet rich fibrin significantly promotes gains in clinical attachment, bone fill and other periodontal supporting structures. The present study was undertaken to compare the efficacy of DFDBA alone with DFDBA in combination with PRF in the treatment of vertical intrabony defects in terms of bony defect fill assessed clinically and radiographically using Cone Beam Computed Tomography scan.

A total of 30 interproximal angular defects in patients suffering with chronic periodontitis in the age range of 35-60 years were selected for the study. They were randomly divided into two groups of 15 sites each, to be treated with DFDBA (Group A) and DFDBA with PRF (Group B). the clinical parameters were assessed at baseline, 3 months and 6 months whereas the radiographic parameters were recorded at baseline and 6 months post treatment.

Favourable clinical outcomes were achieved for both the treatment groups when compared to baseline. Group B demonstrated significantly and comparatively higher bone fill in all the radiographic parameters (increase in Bone height, bone volume gain, defect angle) as compared to Group A.

This study, thus demonstrated that although both the materials are capable to improve the clinical and radiographic parameters in treating interproximal osseous defects, the combination of DFDBA and PRF has comparatively higher ability for the same.

INTRODUCTION

Periodontitis, which progressively destroys tooth-supporting structures, is one of the most widespread infectious diseases and the leading cause of tooth loss in adults.[1] Periodontal treatment including scaling and root planing and Open flap debridement(when indicated) are highly effective at repairing disease related defects and halting the progression of periodontitis. While these are important steps, it is still required to develop more effective techniques that predictably promote the body's natural ability to regenerate its lost periodontal tissues, particularly periodontal ligament and alveolar bone.[2]

Regeneration of lost structures has become the primary therapeutic goal in periodontics and there are numerous therapeutic modalities for restoring periodontal osseous defects that have been investigated.[3]

Many of these procedures include the use of bone grafts and bone replacement materials.[4] A graft is any tissue, organ or material used for implantation or transplantation and to induce union between normally separated tissues (Glossary of Periodontal Terms)

Several types of bone grafts have been studied over the years, and periodontists continue to search for ideal materials. Bone graft materials have osteogenic, osteoinductive and osteoconductive potential.

DFDBA (Demineralized freeze-dried bone allograft) for the last few decades, has been used alone or in combination with other treatment modalities for periodontal regeneration. The current widespread use of DFDBA is based on the professed osteoinductive ability of demineralized graft preparations.[5,6] In the 1960's, Urist discovered that demineralized bone could stimulate

bone formation in soft tissue and named the putative active agent "Bone morphogenetic protein".[7] When implanted into heterotopic host sites, BMP-containing extracts of demineralized bone induce endochondral ossification that terminates in the formation of a complete ossicle containing lamellar bone and bone marrow.[8.9] The presence of bone morphogenetic proteins contained within DFDBA aids in mesenchymal cell migration, attachment, and osteogenesis. DFDBA has both osteoinductive and osteoconductive activity and the ability to create and maintain the space.[10]

Another material that has been used in this study is Platelet Rich Fibrin that is a second-generation platelet concentrate. Platelet rich fibrin (PRF) 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. Platelet-rich fibrin (PRF) was introduced by Choukroun et al. in 2001.[11] PRF is in the form of platelet gel and can be used in conjunction with bone grafts, which offers several advantages, including promoting wound healing, bone growth and maturation, graft stabilization, wound healing, and hemostasis, and improving the handling properties of graft materials.

Hence, considering the above advantages of both DFDBA as well as PRF, the study focused on comparison in the efficacies of these regenerative materials in the treatment of intrabony defects, as DFDBA alone and DFDBA in combination with PRF.

AIM : To evaluate the clinical and radiographic outcomes observed in treating intrabony defects with DFDBA alone and DFDBA in conjunction with PRF.

OBJECTIVES :

1. To evaluate the efficacy of DFDBA for treating angular defects.
2. To assess the efficacy of DFDBA combined with PRF for treating angular defects.
3. To compare the difference in efficacy between the two groups.

MATERIALS AND METHODS:

This clinical, experimental prospective study was carried out in the Department of Periodontics, Babu Banarasi Das College of Dental Sciences (BBDCODS), Lucknow**.** Patients were selected based upon the following inclusion and exclusion criteria.

- ✓ **Inclusion criteria -**

    1. Patients in the age group of 35-60 years

    2. Patients suffering from Chronic Periodontitis with Probing Pocket depth ≥ 6 mm.

    3. Patients with radiographic evidence of intrabony defects.

- ✓ **Exclusion criteria -**

    1. Patients with any systemic diseases that affects the periodontal treatment outcome.
    2. Smokers and tobacco chewers
    3. Subjects with a known allergy to the material being used
    4. Pregnant and lactating women
    5. Patients who have used antibiotics for the previous 3 months.
    6. Non co-operative patients.

- **Materials:**

1. Syringe 3ml and 5ml.
2. Mouth mirrors, UNC-15 Probe (Hu-Freidy)
3. Local anaesthetic agent 2% Lignocaine.
4. A set of surgical curettes
5. BP blade handle, Blade No. 12, 15, Periosteal elevator (Hu-friedy)
6. Demineralized freeze-dried bone allograft (DFDBA) from Rocky Mountain Tissue Bank provided in cancellous particulate vials as Irradiated Allogenic Cancellous Bone and Marrow Particulate - Randomly sized 2-3 mm particles.
7. Platelet Rich Fibrin (PRF)
8. Cumine scaler and condensor.
9. Adams tissue holding forceps.
10. Castroviejo scissors, needle and holder.
11. Sutures(4-0) non-resorabable braided silk.
12. Laboratory centrifuge(Forco scientific Udyog Pvt.Ltd)
13. Coe-pack dressing.

- **Study Design:**

The treatment procedure was fully explained to the patients after taking the institutional ethical clearance. A duly signed consent form was taken from each patient before initiating the treatment. 30 sites fulfilling the inclusion and exclusion criteria were selected and all the sites were than randomly distributed into two groups viz. Group A and Group B.

- ✓ Group A – intrabony defects treated with DFDBA alone
- ✓ Group B – intrabony defects treated with DFDBA in combination with PRF

Further these individuals were subjected to Cone Beam Computed Tomography scan (CBCT) that was done at the Department of Oral Medicine and Radiology, KGMU. NewTom Cone Beam 3D imaging unit was used for this purpose. Over other imaging modalities, CBCT was preferred as it provided multiple sections of the dental anatomy with considerably lower radiation exposure. So, this would provide near to accurate changes in bone morphology with multiple fields of view and higher resolution and help authenticate the study.

- **Methodology:**

At Baseline, the following clinical and radiographic parameters were recorded:

- **Clinical parameters**

- Gingival Index - GI (Loe and Silness, 1963)
- Plaque Index - PI (Silness and Loe, 1964)
- Pocket Probing depth - PPD
- Clinical Attachment Level – CAL

❖ **Radiographic evaluation**

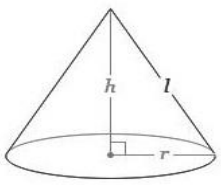
Abbreviations used (considering the defect morphology roughly close to that of a cone):

- FRP – fixed reference point – taken as CEJ of the affected tooth
- BD – base of the defect
- DD – defect depth i.e distance from FRP to BD , also considered as the height of the cone
- RDA – radiographic defect angle : intersection of two lines that represent the root surface of the involved tooth and the bone defect surface.[12] (at the BD)
- D – diameter of the cone : the horizontal distance from alveolar crest (AC)/coronal point of the defect to the tooth involving the defect, at FRP.
- V – volume of cone : $\pi r^2 h/3$, where h = DD

$$V = \pi r^2 \frac{h}{3}$$

r Radius

h Height



- ✓ **Surgical procedure:**

All the subjects included in the study underwent Phase I therapy and were recalled after one month for surgical intervention. All the clinical and radiographic parameters were recorded as Baseline readings. After the recordings, they were asked for a pre-procedural rinse with 10ml of 0.2% chlorhexidine gluconate solution for 2 minutes. The surgical procedure was performed under aseptic conditions. The operative sites were anesthetized with a solution of 2% lignocaine with 1:200,000 adrenaline. Sulcular incisions were given and full thickness flap was reflected. The surgical area was then irrigated with sterile saline and was carefully inspected to ensure the debridement completion.

The defect sites in Group A were grafted with DFDBA. The graft was placed and condensed into the defect. Care was taken to avoid the overfilling of the defect so as to ensure adequate closure of the flap. Also, over-condensation was avoided for sufficient vascularization within the graft and to prevent any infection. The flap was sutured in close approximation using interrupted sutures. Surgical site was protected by applying a periodontal dressing. (Plate no.

Similar surgical procedure was done for Group B. The sites were grafted with DFDBA in combination with PRF. To obtain PRF, 10 ml blood was drawn from the median cubital vein from the cubital fossa and was placed in sterilized test tubes without anticoagulant and centrifuged immediately at 3000 rpm for 10 min using the centrifuge. (Forco scientific Udyog Pvt.Ltd). The resultant product consisted of the following three layers: (Plate No.)

1. Topmost layer - Acellular platelet poor plasma
2. Middle – Platelet rich fibrin (PRF)
3. Bottom layer - Red blood corpuscles.

Following this, the PRF clot was retrieved alongwith the associated RBC layer with tweezers from the test tubes. The RBC layer just below PRF/RBC junction was cut using scissors. The PRF clot was then placed on a glass slab over a gauge piece and gently compressed using another glass slab to remove excess serum. [13]

The platelet rich fibrin was used as a membrane in the sites under this category. This PRF membrane was placed over the DFDBA graft followed by repositioning of the soft tissue flaps at the original level, closed with interrupted sutures. A tension free primary closure of the flaps was achieved and the surgical site was protected by applying a periodontal dressing.

Antibiotics and analgesics were prescribed for both the groups. Patients were recalled after 10 days for suture removal, dressing removal and examination. Plaque control was reinforced at the time of suture removal. Further recalls for clininal re-evaluation were scheduled at 3 months and 6 months. Radiographic evaluation was done 6 months post-operatively. At each visit, plaque control measures were reinforced and supra gingival scaling was done if required.

At the end of the study, the entire data thus collected was subjected to suitable statistical analysis and interpretation for final results.

OBSERVATIONS AND RESULTS

Statistical analysis

The results are presented in mean±SD. The Unpaired t-test was used to compare the study parameters between groups at baseline, 3 months and 6 months. The Paired t-test was used to compare the mean change in the study parameters from baseline to 3 and 6 months within the groups. The p-value<0.05 was considered statistically significant. All the analysis was carried out on SPSS 16.0 version (Chicago, Inc., USA).

Clinical parameters

I. Comparison of PI between Group A and Group B at Baseline, 3 months and 6 months (post-operatively)

Inter-Group:

Plaque index scores were recorded at these time intervals in both the groups.

At Baseline, the mean PI readings for Group A was 0.68±0.12 and Group B was 0.72±0.12. The p-value difference between both the groups was 0.23, that was statistically non-significant.

3 months post-operatively, the mean PI readings for Group A was 0.64±0.11and Group B was 0.61±0.10. The p-value difference between both the groups was 0.09, that was statistically non-significant.

6 months post-operatively, the mean PI readings for Group A was 0.62±0.13 and Group B was 0.58±0.09. The p-value difference between both the groups was 0.13, that was statistically non-significant.

It was also observed that PI scores were lesser in Group B as compared to Group A, however, the difference was not statistically significant. (Table 1a and Fig 1a)

Table-1a: Inter-group comparison of PI between the groups at baseline, 3 and 6 months

Time periods	Group A (n=15)	Group B (n=15)	p-value[1]	Statistical significance
Baseline	0.68±0.12	0.72±0.12	0.23	NS
3 months	0.64±0.11	0.61±0.10	0.09	NS
6 months	0.62±0.13	0.58±0.09	0.13	NS

[1]Unpaired t-test, S=significant, NS=non-significant, $p<0.05$

Intra-Group:

In group A, the mean plaque index at baseline was 0.68±0.12, that reduced to 0.64±0.11 after 3 months, showing a reduction of 0.04±0.01. This change was found to be statistically non-significant.(p=0.085)

The mean plaque index at baseline was 0.68±0.12, that reduced to 0.62±0.13 after 6 months, showing a reduction of 0.06±0.02. This change was found to be statistically non-significant.(p=0.08)

In group B, the mean plaque index at baseline was 0.72±0.12, that reduced to 0.61±0.10 after 3 months, showing a reduction of 0.11±0.07. This change was found to be statistically no-significant.(p=0.06)

The mean plaque index at baseline was 0.72±0.12, that reduced to 0.58±0.09 after 6 months, showing a reduction of 0.14±0.08. This change was found to be statistically non-significant.(p=0.06)

Table-1b: Intra-group comparison of PI between the groups at baseline, 3 and 6 months

Groups	Baseline	3 months	6 months	p-value[1]	Statistical significance
Group A	0.68±0.12	0.64±0.11	0.62±0.13	0.08	NS
Group B	0.72±0.12	0.61±0.10	0.58±0.09	0.06	NS

[1]Unpaired t-test, S=significant, NS=non-significant, p<0.05

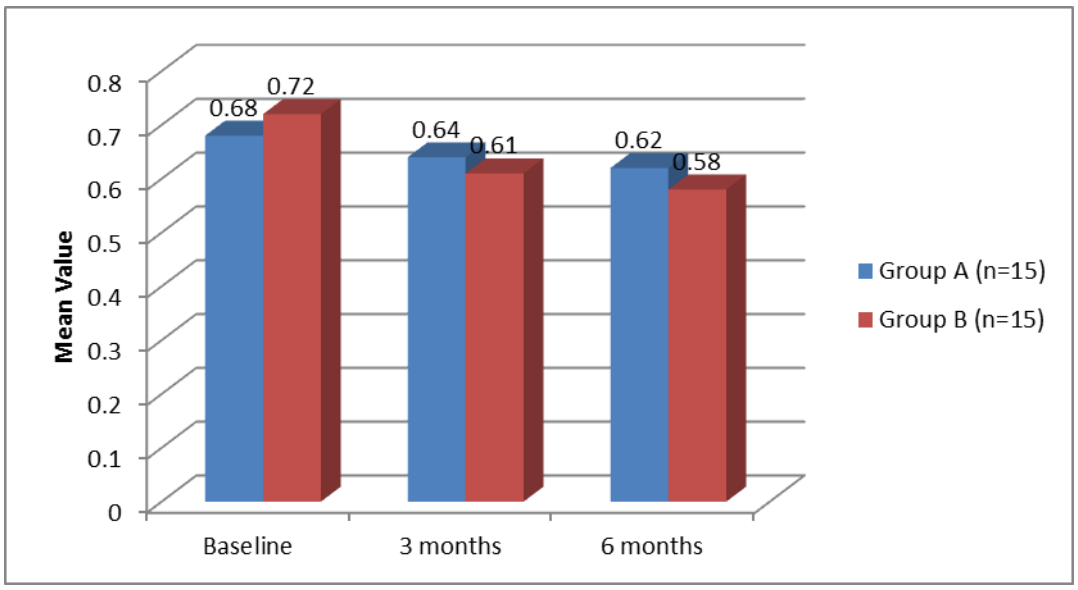


Fig. 1a: Inter-group comparison of PI between Group A and Group B at baseline, 3 and 6 months

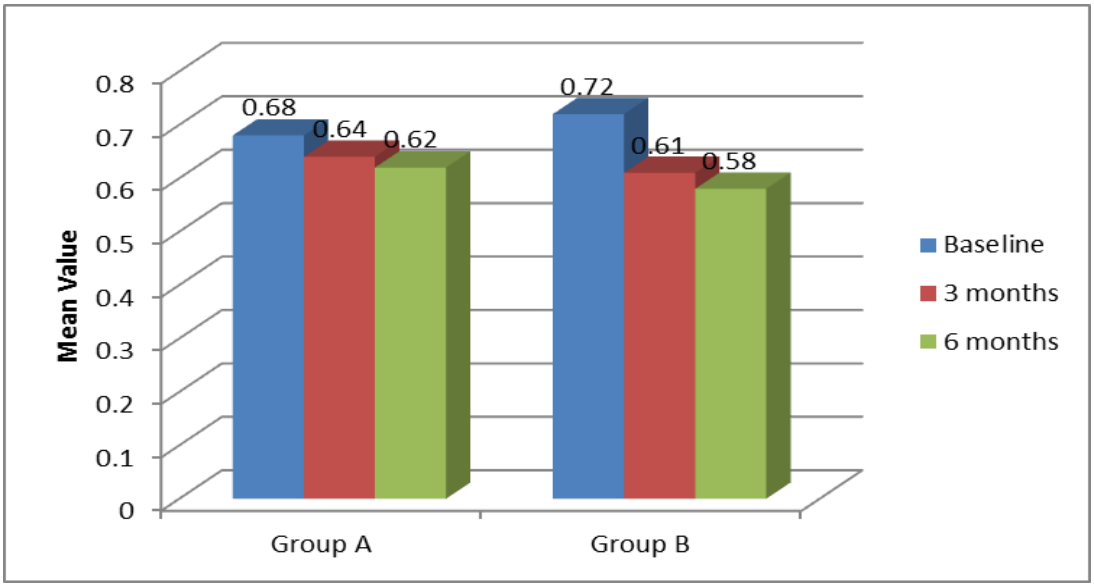


Fig. 1b: Intra-group comparison of PI between Group A and Group B at baseline, 3 and 6 months

II. Comparison of GI between Group A and Group B at Baseline, 3 and 6 months (post-operatively)

Inter-Group:

Gingival index scores were recorded at these time intervals in both the groups.

At Baseline, the mean GI readings for Group A was 0.64±0.14 and Group B was 0.63±0.16. The p-value difference between both the groups was 0.11, that was statistically non-significant.

3 months post-operatively, the mean GI readings for Group A was 0.63±0.11and Group B was 0.62±0.12. The p-value difference between both the groups was 0.12, that was statistically non-significant.

6 months post-operatively, the mean GI readings for Group A was 0.61±0.08 and Group B was 0.60±0.09. The p-value difference between both the groups was 0.17, that was statistically non-significant.

It was also observed that GI scores were lesser in Group B as compared to Group A, however, the difference was not statistically significant. (Table 2a and Fig 2a)

Table-2a: Inter-group comparison of GI between Group A and Group B at Baseline, 3 and 6 months

Time periods	Group A (n=15)	Group B (n=15)	p-value[1]	Statistical significance
Baseline	0.64±0.14	0.63±0.16	0.11	NS
3 months	0.63±0.11	0.625±0.12	0.12	NS
6 months	0.61±0.08	0.605±0.09	0.17	NS

[1]Unpaired t-test, S=significant, NS=non-significant, $p<0.05$

Intra-Group:

In group A, the mean gingival index at baseline was 0.64±0.14, that reduced to 0.63±0.11 after 3 months, showing a reduction of 0.01±0.01. This change was found to be statistically non-significant.(p=0.085)

The mean gingival index at baseline was 0.64±0.14, that reduced to 0.61±0.08 after 6 months, showing a reduction of 0.03±0.09. This change was found to be statistically non-significant.(p=0.08)

In group B, the mean gingival index at baseline was 0.63±0.16, that reduced to 0.62±0.12 after 3 months, showing a reduction of 0.12±0.07. This change was found to be statistically non-significant.(p=0.06)

The mean gingival index at baseline was 0.63±0.16, that reduced to 0.60±0.09 after 6 months, showing a reduction of 0.03± 0.08. This change was found to be statistically significant.(p=0.04)

This can be observed in Table 2a and Fig 2b.

Table-2b: Intra-group comparison of GI between the groups at baseline, 3 and 6 months

Groups	Baseline	3 months	6 months	p-value[1]	Statistical significance
Group A	0.64±0.14	0.63±0.11	0.61±0.08	0.08	NS
Group B	0.63±0.16	0.625±0.12	0.605±0.09	0.04	S

[1]Unpaired t-test, S=significant, NS=non-significant, $p<0.05$

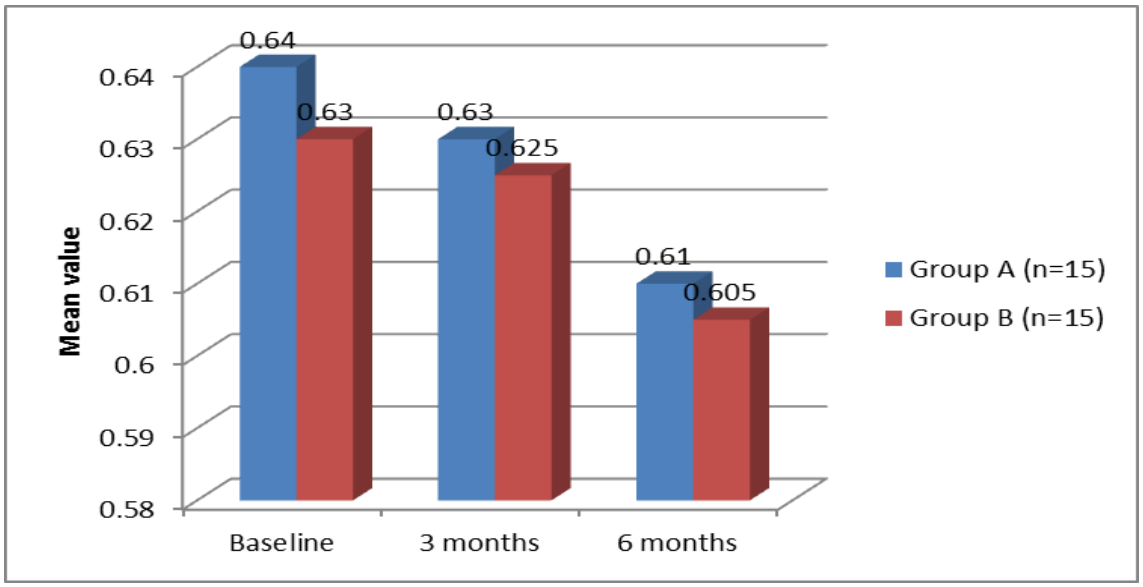


Fig. 2a: Inter-group comparison of GI between the groups at baseline, 3 and 6 months

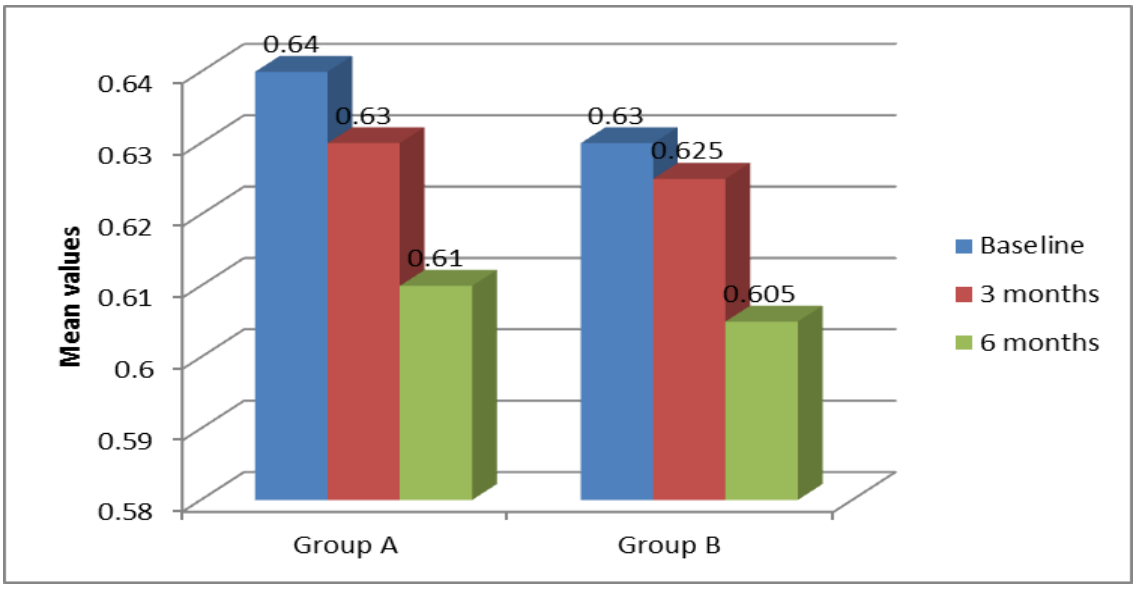


Fig. 2b: Intra-group comparison of GI between the groups at baseline, 3 and 6 months

III. Comparison of CAL reduction (attachment gain) between Group A and Group B at Baseline, 3 months and 6 months (post-operatively)

Inter-Group:

CAL scores were recorded at these time intervals in both the groups.

At Baseline, the mean CAL readings for Group A was 7.49±2.23 and Group B was 7.26±3.12. The p-value difference between both the groups was 0.18, that was statistically non-significant.

3 months post-operatively, the mean CAL readings for Group A was 5.88±2.14 and Group B was 5.94±2.23. The p-value difference between both the groups was 0.11, that was statistically non-significant.

6 months post-operatively, the mean CAL readings for Group A was 5.81±1.98 and Group B was 4.96±2.13. The p-value difference between both the groups was 0.14, that was statistically non-significant.

It was also observed that CAL scores were higher in Group B as compared to Group A, however, the difference was not statistically significant.(Table 3a and Fig 3a)

Table-3a: Comparison of CAL between the groups across the time periods

Time periods	Group A (n=15)	Group B (n=15)	p-value[1]	Statistical significance
Baseline	7.49±2.23	7.26±3.12	0.18	NS
3 months	5.88±2.14	5.94±2.23	0.11	NS
6 months	5.81±1.98	4.96±2.13	0.14	NS

[1]Unpaired t-test, S=significant, NS=non-significant, $p<0.05$

Intra-Group:

In group A, the mean CAL at baseline was 7.49±2.23, that reduced to 5.88±2.14 after 3 months, showing a reduction of 1.61±0.01. This change was found to be statistically significant.(p=0.05)

The mean CAL at baseline was 7.49±2.23, that reduced to 5.81±0.13 after 6 months, showing a reduction of 1.68±0.02. This change was found to be statistically significant.(p=0.048)

In group B, the mean CAL at baseline was 7.26±3.12, that reduced to 5.94±2.23 after 3 months, showing a reduction of 1.32± 0.07. This change was found to be statistically significant.(p=0.04)

The mean CAL at baseline was 7.26±3.12, that reduced to 4.96±2.13 after 6 months, showing a reduction of 2.30± 0.08. This change was found to be statistically significant.(p=0.035)

This can be observed in the Table 3b and Fig 3b.

Table-3b: Intra-group comparison of CAL between the groups at baseline, 3 and 6 months

Groups	Baseline	3 months	6 months	p-value[1]	Statistical significance
Group A	7.49±2.23	5.88±2.14	5.81±1.98	0.04	S
Group B	7.26±3.12	5.94±2.23	4.96±2.13	0.03	S

[1]Unpaired t-test, S=significant, NS=non-significant, $p<0.05$

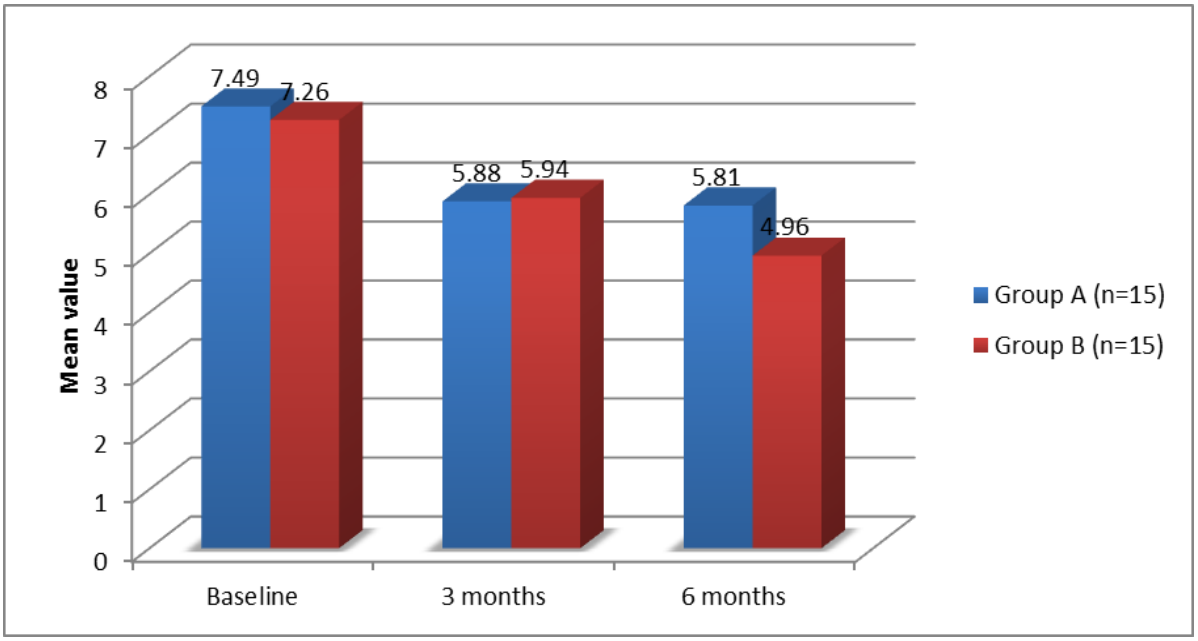


Fig. 3a: Inter-group comparison of CAL between Group A and Group B at baseline, 3 and 6 months

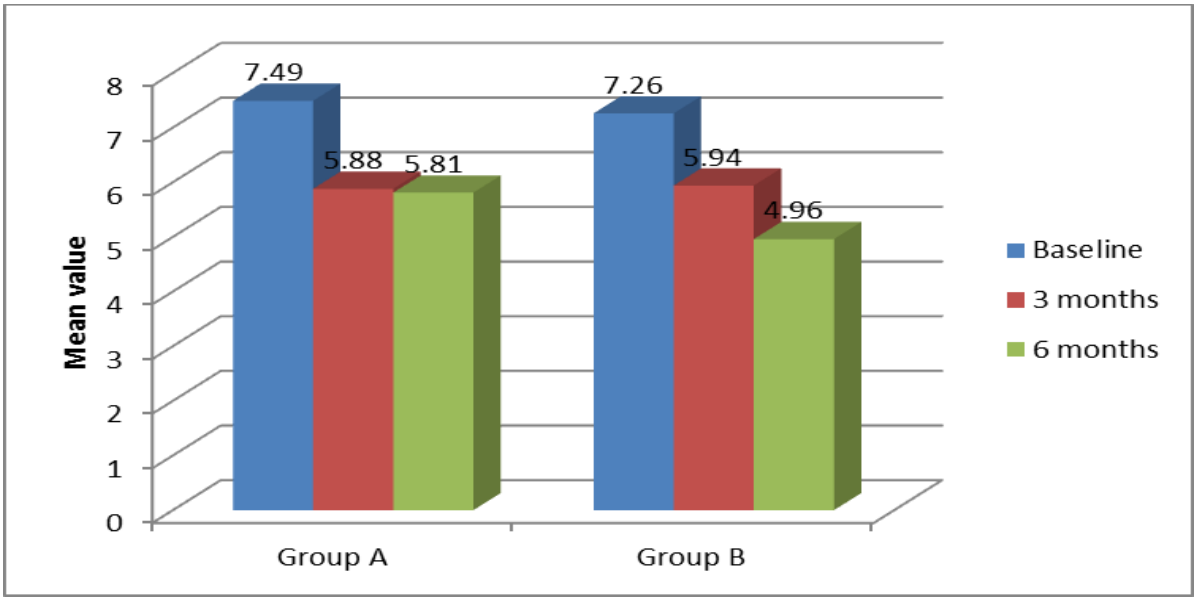


Fig. 3b: Intra-group comparison of CAL between Group A and Group B at baseline, 3 and 6 months

IV. Comparison of Probing pocket depth between Group A and Group B at Baseline, 3 months and 6 months

Inter-Group:

PPD scores were recorded at these time intervals in both the groups.

At Baseline, the mean PPD readings for Group A was 7.83±3.12 and Group B was 8.12±3.11. The p-value difference between both the groups was 0.17, that was statistically non-significant.

3 months post-operatively, the mean PPD readings for Group A was 5.91±2.14 and Group B was 5.41±2.13. The p-value difference between both the groups was 0.29, that was statistically non-significant.

6 months post-operatively, the mean PPD readings for Group A was 4.27±1.14 and Group B was 2.92±0.98. The p-value difference between both the groups was 0.06, that was statistically non-significant.

It was also observed that PPD scores were lesser in Group B as compared to Group A, which is statistically non-significant.(Table 4a and Fig 4a)

Table-4a: Inter-group comparison of PPD between Group A and Group B at baseline, 3 months and 6 months

Time periods	Group A (n=15)	Group B (n=15)	p-value[1]	Statistically significant
Baseline	7.83±3.12	8.12±3.11	0.17	NS
3 months	5.91±2.14	5.41±2.13	0.29	NS
6 months	4.27±1.14	2.92±0.98	0.06	NS

[1]Unpaired t-test, S=significant, NS=non-significant, $p<0.05$

Intra-Group:

In group A, the mean PPD at baseline was 7.83±3.12, that reduced to 5.91±2.14 after 3 months, showing a reduction of 1.92±0.01. This change was found to be statistically significant.(p=0.05)

The mean PPD at baseline was 7.83±3.12, that reduced to 4.27±1.14 after 6 months, showing a reduction of 3.56±0.02. This change was found to be statistically significant.(p=0.04)

In group B, the mean PPD at baseline was 8.12±3.11, that reduced to 5.41±2.13 after 3 months, showing a reduction of 2.71± 0.07. This change was found to be statistically significant.(p=0.03)

The mean PPD at baseline was 8.12±3.11, that reduced to 2.92±0.98 after 6 months, showing a reduction of 5.20±0.08. This change was found to be statistically significant.(p=0.02)

his can be observed in the Table 4 and Fig 4.

Table-4b: Intra-group comparison of PPD between the groups at baseline, 3 and 6 months

Groups	Baseline	3 months	6 months	p-value[1]	Statistical significance
Group A	7.83±3.12	5.91±2.14	4.27±1.14	0.04	S
Group B	8.12±3.11	5.41±2.13	2.92±0.98	0.03	S

[1]Unpaired t-test, S=significant, NS=non-significant, p<0.05

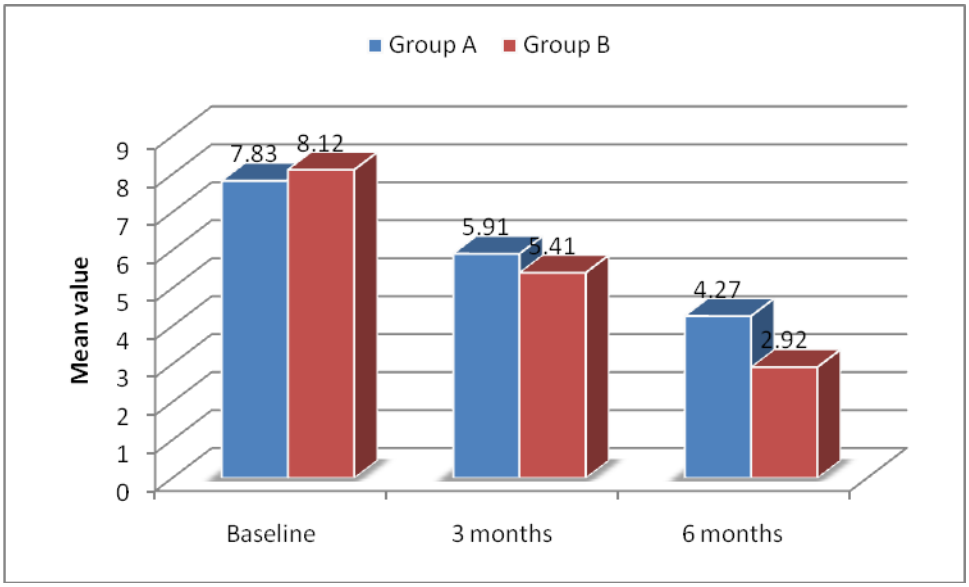


Fig. 4: Inter-group comparison of PPD between Group A and Group B at baseline, 3 and 6 months

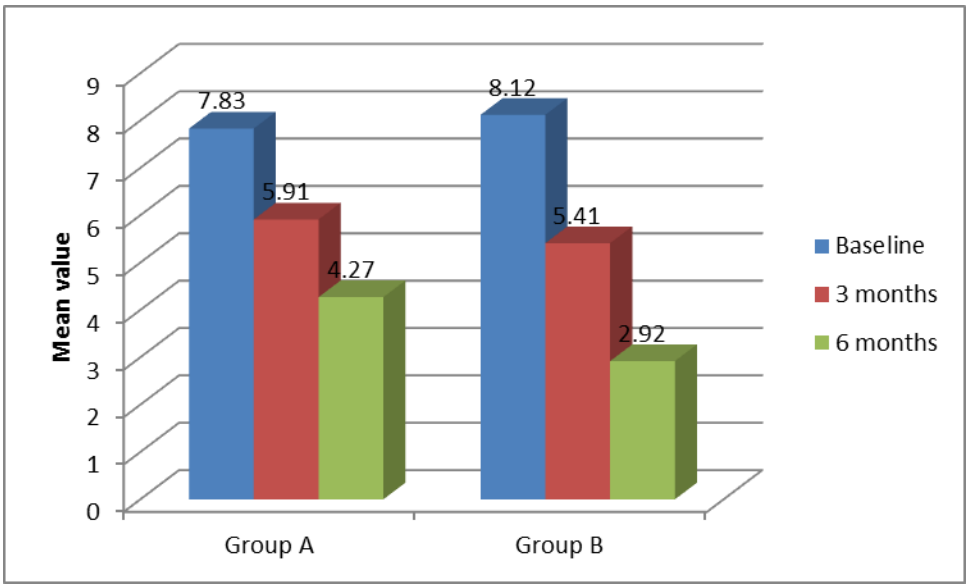


Fig. 4b: Intra-group comparison of PPD between Group A and Group B at baseline, 3 and 6 months

V. Comparison of reduction in defect depth Group A and Group B at baseline and 6 months

Inter-Group:

At Baseline, the mean defect depth readings for Group A was 7.22±3.12 and Group B was 8.16±3.25. The p-value difference between both the groups was 0.29, that was statistically non-significant.

6 months post-operatively, the mean defect depth readings for Group A was 5.26±2.15 and Group B was 4.89±1.11. The p-value difference between both the groups was 0.11, that was statistically non-significant.

It was also observed that defect depth reduction was higher in Group B (3.27±0.97) as compared to Group A (1.96±0.57), which is statistically significant. (p=0.02) (Table 5a and Fig 5a)

Table-5a: Comparison of reduction in defect depth between Group A and Group B at baseline and 6 months

Time periods	Group A (n=15)	Group B (n=15)	p-value[1]	Statistical significance
Baseline	7.22±3.12	8.16±3.25	0.29	NS
6 months	5.26±2.15	4.89±1.11	0.11	NS

[1]Unpaired t-test, S=significant, NS=non-significant, $p<0.05$

Intra-Group:

In group A, the mean defect depth at baseline was 7.22±3.12, that reduced to 5.26±2.15 after 6 months, showing a reduction of 1.96±0.57. This change was found to be statistically significant.(p=0.01*)

In group B, the mean defect depth reduction at baseline was 8.16±3.25, that reduced to 4.89±1.11 after 6 months, showing a reduction of 3.27±0.97. This change was found to be statistically significant.(p=0.001*)

This can be observed in the Table 5b and Fig 5b.

Table-5b: Intra-group comparison of reduction in defect depth between the groups at baseline, 3 and 6 months

Groups	Baseline	6 months	p-value[1]	Statistical significance
Group A	7.22±3.12	5.26±2.15	0.01*	S
Group B	8.16±3.25	4.89±1.11	0.001*	S

[1]Unpaired t-test, S=significant, NS=non-significant, p<0.05

Table. 5c: Comparison of reduction difference in defect depth between Group A and Group B from baseline to 6 months

Time periods	Group A (n=15)	Group B (n=15)	p-value[1]	Statistical significance
Reduction difference in Defect depth	1.96±0.57	3.27±0.97	0.02	S

[1]Unpaired t-test, S=significant, NS=non-significant, p<0.05

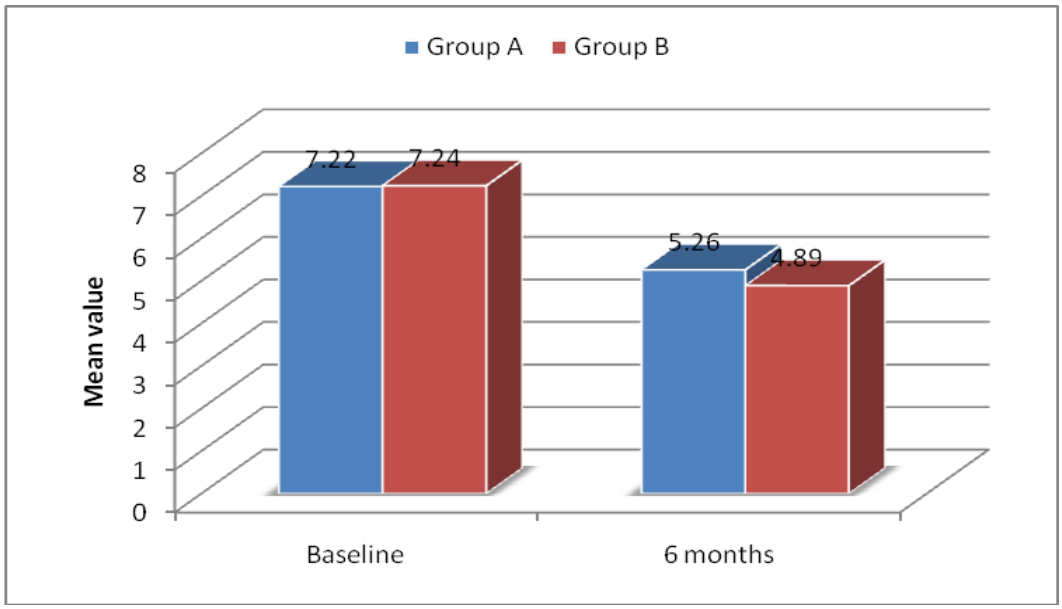


Fig. 5a: Inter-group comparison of reduction in defect depth between Group A and Group B at baseline and 6 months

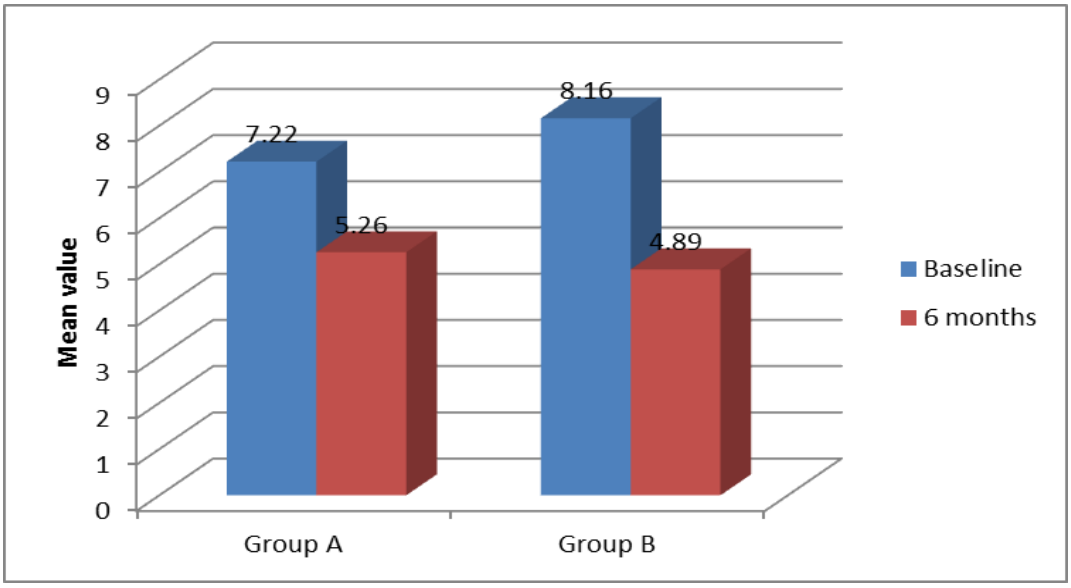


Fig. 5b: Intra-group comparison of reduction in defect depth between Group A and Group B at baseline and 6 months

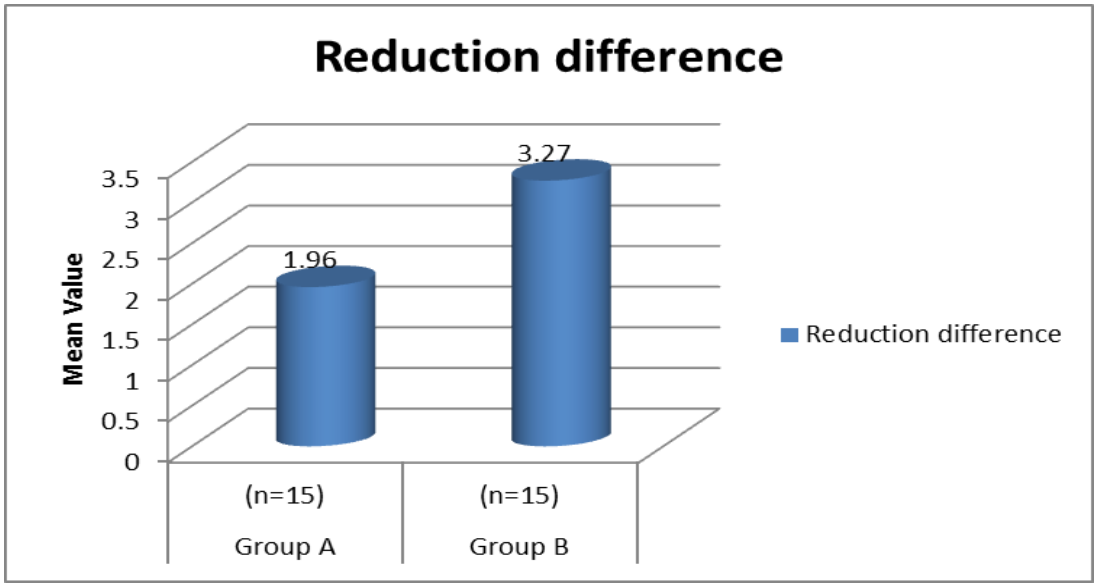


Fig. 5c: Comparison of reduction difference in defect depth between Group A and Group B from baseline to 6 months

VI. Comparison of volumetric bone gain between Group A and Group B at baseline and 6 months

Inter-Group:

At Baseline, the mean volume readings for Group A was 75.79±3.12 and Group B was 90.57±3.11. The p-value difference between both the groups was 0.01, that was statistically significant.

6 months post-operatively, the mean volume readings for Group A was 37.25±2.14 and Group B was 41.02±2.13. The p-value difference between both the groups was 0.02, that was statistically significant.

It was seen that the volumetric bone gain was pronounced and statistically significant in Group B (49.55±3.56) as compared to Group A(38.54±2.17). (p=0.001*) (Table 6a and Fig 6a)

Table-6a: Inter-group comparison of volumetric bone gain between the groups at baseline and 6 months

Time periods	Group A (n=15)	Group B (n=15)	p-value[1]	Statistical significance
Baseline	75.79±2.71	90.57±3.20	0.001*	S
6 months	37.25±2.24	41.02±1.45	0.001*	S

[1]Unpaired t-test, *Significant, S=significant, NS=non-significant, p<0.05

Intra-Group:

In group A, the mean volume at baseline was 75.79±2.71, that reduced to 37.25±2.24 after 6 months, showing a reduction of 38.54±2.17, that is interpreted as gain in bone volume. This change was found to be statistically significant.(p=0.0001*)

In group B, the mean volume at baseline was 90.57±3.20, that reduced to 41.02±1.45 after 6 months, showing a reduction of 49.55±3.56, that is interpreted as gain in bone volume. This change was found to be statistically significant.(p=0.0001*)

This can be observed in the Table 6b and Fig 6b.

Table-6b: Intra-group comparison of volumetric bone gain between the groups at baseline and 6 months

Groups	Baseline	6 months	p-value[1]	Statistical significance
Group A	75.79±2.71	37.25±2.24	0.0001*	S
Group B	90.57±3.20	41.02±1.45	0.0001*	S

[1]Unpaired t-test, S=significant, NS=non-significant, p<0.05

Table. 6c: Comparison of Bone volume gain difference between Group A and Group B from baseline to 6 months

Time periods	Group A (n=15)	Group B (n=15)	p-value[1]	Statistical significance
Bone volume gain difference	38.54±2.17	49.55±3.56	0.001*	S

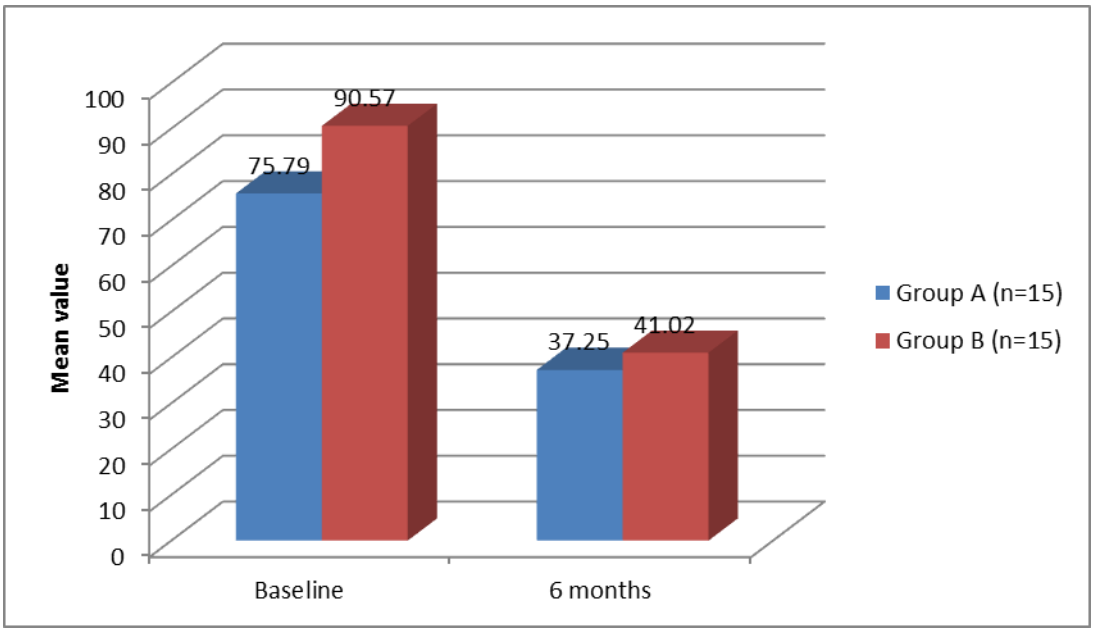


Fig. 6a: Inter-group comparison of volumetric bone gain between Group A and Group B at baseline and 6 months

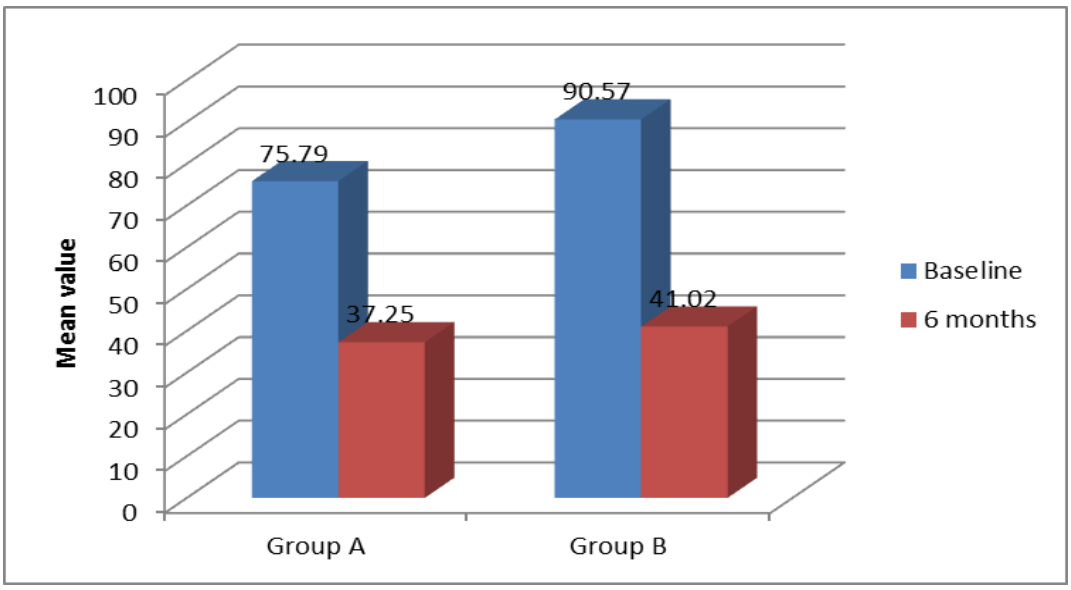


Fig. 6b: Intra-group comparison of volumetric bone gain between Group A and Group B at baseline and 6 months

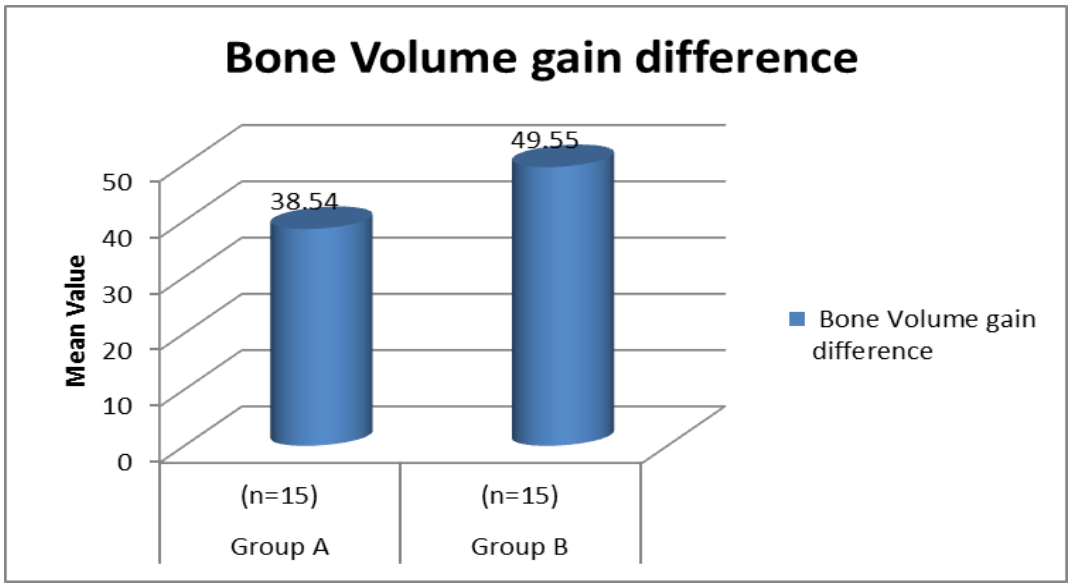


Fig. 6a: Comparison of Bone volume gain difference between Group A and Group B from baseline to 6 months

VII. Comparison of radiographic defect angle between Group A and Group B at baseline and 6 months

Inter-Group:

At Baseline, the mean radiographic defect angle readings for Group A was 42.45±3.12 and Group B was 46.29±3.11. The p-value difference between both the groups was 0.17, that was statistically non-significant.

6 months post-operatively, the mean radiographic defect angle for Group A was 47.00±2.14 and Group B was 66.00±2.13. The p-value difference between both the groups was 0.02, that was statistically significant.

On comparing both the groups after 6 months, the increase in Radiographic defect angle was found to be significantly more in Group B (19.71±0.97) as compared to Group A (4.55±0.57), that was also statistically significant. (p=0.001*) (Table 7a and Fig 7a)

Table-7a: Inter-group comparison of radiographic defect angle between Group A and Group B at baseline and 6 months

Time periods	Group A (n=15)	Group B (n=15)	p-value[1]	Statistical significance
Baseline	42.45±3.41	46.29±3.04	0.11	NS
6 months	47.00±4.23	66.00±2.44	0.001*	S

[1]Unpaired t-test, *Significant, S=significant, NS=non-significant, p<0.05

Intra-Group:

In group A, the mean radiographic defect defect angle at baseline was 42.45±3.12, that increased to 47.00±2.15 after 6 months, showing an increase of 4.55±0.57. This change was found to be statistically significant.(p=0.01*)

In group B, the mean radiographic defect defect angle at baseline was 46.29±3.25, that increased to 66.00±1.11 after 6 months, showing an increase of 19.71±0.97. This change was found to be statistically significant.(p=0.001*)

This can be observed in the Table 7b and Fig 7b.

Table-7b: Intra-group comparison of radiographic defect angle between Group A and Group B at baseline and 6 months

Groups	Baseline	6 months	p-value[1]	Statistical significance
Group A	42.45±3.41	47.00±4.23	0.01*	S
Group B	46.29±3.04	66.00±2.44	0.001*	S

[1]Unpaired t-test, S=significant, NS=non-significant, p<0.05

Time periods	Group A (n=15)	Group B (n=15)	p-value[1]	Statistical significance
RDA increase difference	4.55±0.57	19.71±0.97	0.001*	S

Table. 7c: Comparison of RDA increase difference between Group A and Group B from baseline to 6 months

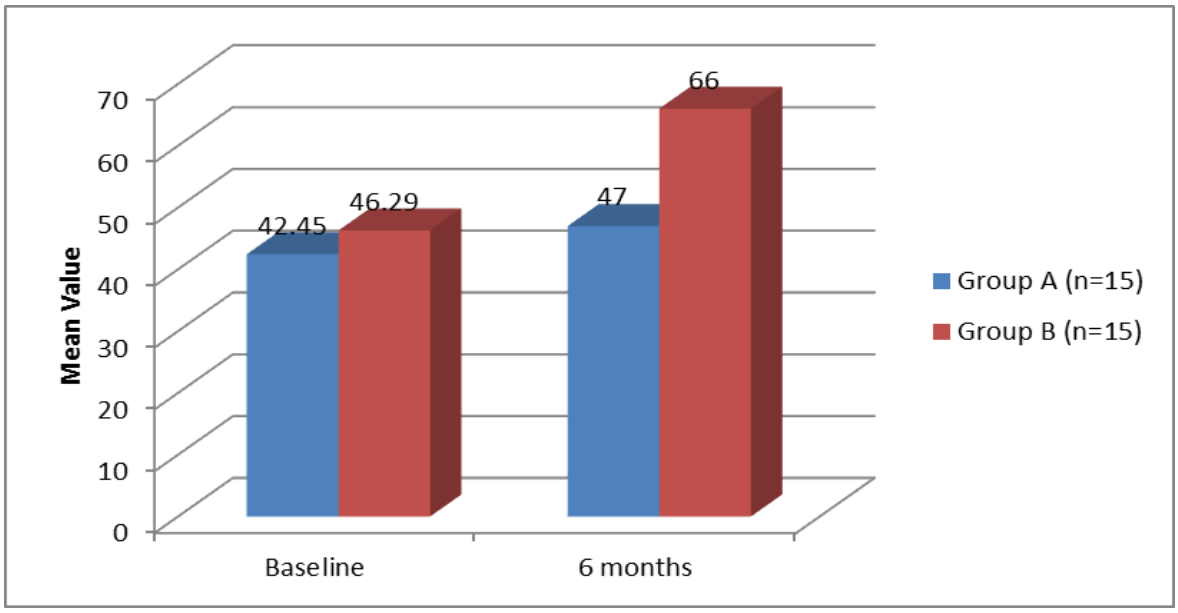


Fig. 7a: Inter-group comparison of radiographic defect angle between Group A and Group B at baseline and 6 months

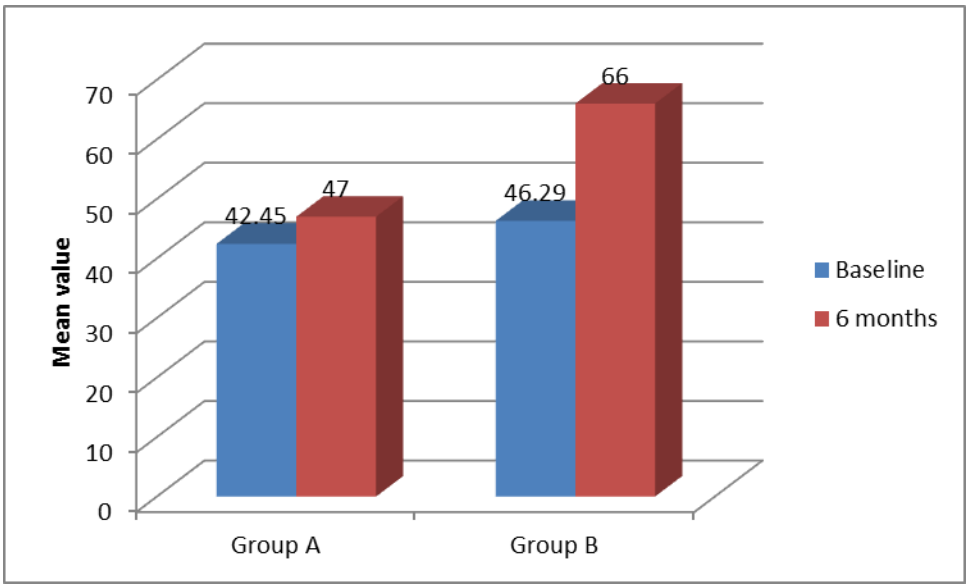


Fig. 7b: Intra-group comparison of radiographic defect angle between Group A and Group B at baseline and 6 months

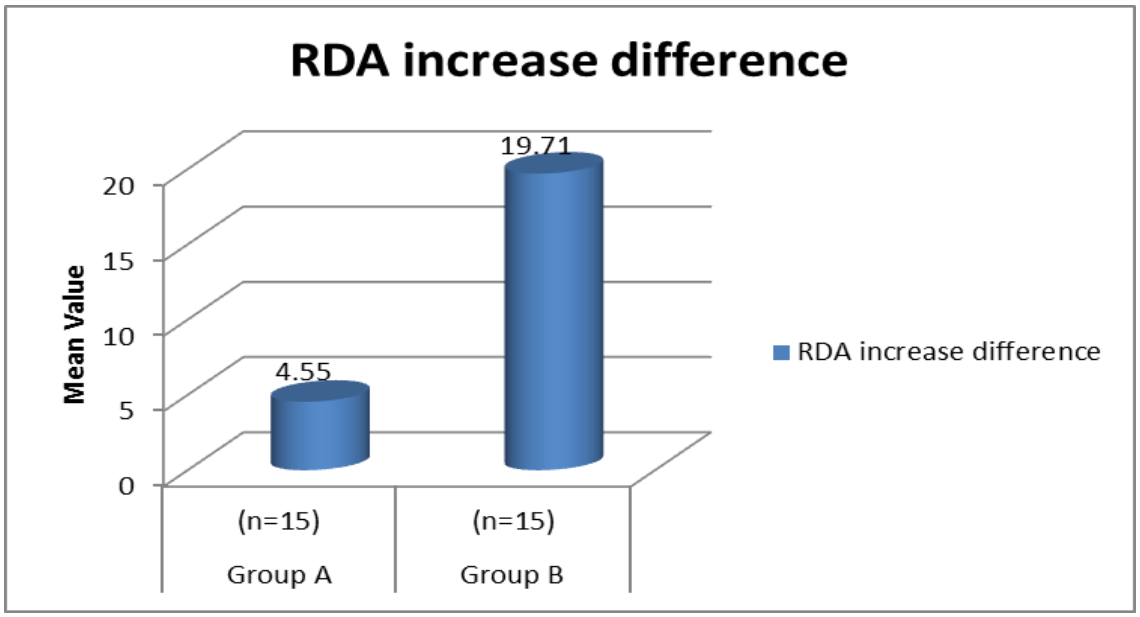


Fig. 7c: Comparison of RDA increase difference between Group A and Group B from baseline to 6 months

DISCUSSION

The following randomized clinical trial was designed to investigate treatment of periodontal Intrabony defects with DFDBA in one Group and DFDBA in combination with PRF in the other Group.

Periodontitis is an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss and bone loss. [53]The height and density of the alveolar bone are normally maintained by an equilibrium, regulated by local and systemic influences, between bone formation and bone resorption.[54,55] When there is an extension of inflammation onto the alveolar bone, bone resorption exceeds bone formation leading to reduction in both bone height and bone density. With increasing age, it becomes more severe because of an accumulation of destruction.[56]

The primary goal of periodontal therapy is to arrest the progression of periodontal disease and maintain the natural dentition in a state of health and comfortable function. However, the ideal outcome is to regenerate the lost periodontal tissues to a prediseased state as far as possible.[61] Although, Regeneration is a significant challenge in periodontal therapy, it seeks to eliminate periodontal defect by forming new bone, along with new cementum and periodontal ligament. Despite difficulties obtaining complete periodontal regeneration of intrabony defects, studies have shown improved clinical parameters using graft materials alone or in combination.[62-66]

Graft materials used for periodontal regeneration can be classified as Autografts, Allografts and Xenografts.

Of these, Autografts and Allografts, Demineralized freeze-dried bone allograft (DFDBA) have histologic evidence of periodontal regeneration in humans.[67-69] Harvesting autogenous bone for

periodontal treatment of periodontal intrabony defect is a cumbersome procedure, also requiring a second surgical site.

DFDBA, used in this study is a common alternative because of its osteo-inductive potential from bone morphogenetic protein exposure during the demineralization process. [70,71]

In the 1960's, Urist discovered that demineralized bone could stimulate bone formation in soft tissue and named the putative active agent "bone morphogenetic protein". [72,73] When implanted into heterotopic host sites, BMP containing extracts of demineralized bone induced endochondral ossification and showed histological evidence of the formation of a new attachment apparatus in infrabony defects.[74] When used with membranes, DFDBA has been shown to promote complete furcation fill in Class II and Class III defects.[75] DFDBA has also been used in defects adjacent to dental implants to promote bone growth[76-80] and for localized alveolar ridge augmentation.[81]

Platelet rich fibrin is an autologous immune second generation platelet concentrate, prepared by a specific centrifugation protocol to allow natural fibrin polymerization. The fibrin network permits aggregation of platelets to form a platelet plug which is favourable for healing and immunity.

PRF is an autologous, immune and platelet concentrate collecting on a single fibrin membrane, containing all the constituents of blood sample which are favorable to healing and immunity.[88]

Retention of platelets and leucocytes within the vast fibrin network allows slow release of growth factors(transforming growth factor - β1, Platelet derived growth factor - AB, Vascular endothelial growth factors) as well as a coagulation glycoprotein (thrombospondin1) during a 7-day course in vitro.[89]

It can be used in conjunction with bone grafts, which has several advantages, such as promoting wound healing, bone growth and maturation, wound sealing and hemostasis, and imparting better handling properties to graft materials.[90,91]

Mazor et al stated that use of PRF as the sole filling material during a simultaneous sinus lift and implantation procedure had stabilized a good amount of regenerated bone in the subsinus cavity upto the tip of implants through a radiological and histological evaluation after 6 months of surgery.[92]

A study by Shetty S in 2009 concluded that treatment of intrabony defects by autologous PRP alone caused significant soft tissue clinical improvement as well as hard tissue defect fill as evidenced by SSD view in spiral computed tomography.[93]

A study by Sarkar et al evaluated bone formation in a long bone defect using a platelet-rich plasma-loaded collagen scaffold and suggested that intrabony defects are known to have higher regenerative potential comparatively.[94]

The Cone beam computed tomography (CBCT) is a digital and mathematical imaging technique that quantifies the intrabony defects in three dimensions. The reformatted CBCT images using NewTom NNT display multiple panoramic and cross-sectional images that assess 3D changes in periodontal osseous defects accurately to the nearest of 0.1mm. Also, CBCT has superiority in evaluating the underlying bony changes. Hence, in the present study we have used CBCT scan to measure 3D changes in the periodontal angular bone defects as well as change in defect depth gain, volumetric bone gain and defect depth angle.[51,95]

This study was designed to investigate treatment of periodontal intrabony defects with only DFDBA in one Group and DFDBA in combination with PRF in the other Group. Results revealed that both treatment modalities resulted in significant improvements in hard and soft

tissue measurements. There were no significant differences in clinical or radiographic outcomes between the two treatment groups.

Clinical Parameters:

The clinical parameters changes of Group A and Group B at 3 months and 6 months are discussed as follows: The mean plaque index decreased by 0.04±0.01 at 3 months, and 0.06±0.02 at 6 months after surgery. The mean gingival index decreased by 0.01±0.01 at 3 months and 0.03±0.09 at 6 months. Similar results were found in Group B from baseline to 3 months and 6 months, reduction in PI was 0.06±0.02 and 0.11±0.07 respectively. Reduction in GI was 0.03±0.09 and 0.12±0.07 at 3 and 6 months respectively. Also, no significant inter-group difference was observed between these two groups. The change in both the parameters that is PI and GI was statistically non- significant. This suggested that the patient maintenance was satisfactory throughout the study in both the groups. Patient compliance is an important factor for determination of prognosis which was good in all patients.

The mean reduction in PPD was 1.92±0.01 after 3 months and 3.56±0.02 after 6 months of surgery in Group A. Similarly, in Group B the reduction in PPD was 2.71±0.07 and 5.20±0.08 after 3 and 6 months respectively. Both the groups showed a significant reduction in PPD from baseline. However, there was no statistically significant reduction difference in PPD when Group B was compared to Group A. This reduction in probing pocket depth in both the groups can be contributed to soft and hard tissue improvements following resolution of inflammation and to the osteogenic potential of the materials used in the study. Our results are in agreement to the

previous studies done using either of these materials who had reported significant reduction in PPD.[82,83]

This is similar to the findings of Shah M et al **who** stated that Platelet-rich fibrin has shown significant results after 6 months, which is comparable to DFDBA for periodontal regeneration in terms of clinical parameters (Probing depth, attachment level). Hence, it can be used in the treatment of intrabony defects.[85]

A study by Hoidal MJ concluded that DFDBA was safe and effective for periodontal defects when the results were observed for Probing depth, Gingival recession and Clinical attachment level.[22]

The mean reduction in CAL was 1.61±0.01 after 3 months and 1.68±0.02 after 6 months of surgery in Group A. Similarly, in Group B the reduction in CAL was 1.32±0.07 and 2.30±0.08 after 3 and 6 months respectively. Both the groups showed a significant reduction in CAL from baseline. However, there was no statistically significant reduction difference in CAL when Group B was compared to Group A.

This is in accordance with the findings of Patel J et al who stated that Platelet-rich fibrin has shown significant results after 6 months, which is comparable to DFDBA for periodontal regeneration in terms of clinical parameters. Hence, it can be used in the treatment of intrabony defects showing significant gain in clinical attachment from baseline.[84]

Our study is in accordance with a similar study done by Blaggana V in 2014, where it was concluded that when DFDBA was used alone in treating human infrabony periodontal defects, CAL gain gain was found to be enhanced significantly.[24]

Bowers et al in a study in 1991 concluded that DFDBA significantly formed new attachment apparatus, thereby, enhancing the regeneration of human intrabony defects.[18]

Juan et al used platelet gel biotechnology in combination with DFDBA and showed a significant reduction in the probing depth and new bone formation, which was evident and was confirmed by radiographs and surgical reentry.[86]

Radiographic parameters:

The mean reduction in defect depth was 1.96±0.57 after 6 months of surgery in Group A. Similarly, in Group B the reduction in defect depth was 3.27±0.97 after 6 months. Both the groups showed a significant reduction in defect depth from baseline. Also, there was statistically significant reduction in defect depth measurements in Group B when compared to Group A (p=0.02)

It was similar to a study by Bowers et al where they reported complete regeneration with new cementum, PDL and bone amounting to 80% of the original defect depth observed at sites treated with DFDBA. New attachment (alveolar bone, cementum and a functional PDL) was observed in intrabony defects where DFDBA was grafted.[16]

In another study by Libin et al it was seen that DFDBA in humans achieved 65% of the bone fill. The implantation of both cortical and cancellous types of allograft resulted in new bone formation and a gain in attachment level.[1]

Aspriello SD in 2011 stated that whether DFDBA was used alone or in combination, both clinical as well as radiographic parameters including hard tissue fill and bone depth reduction, were improved when observed after a period of 6 months.[23]

Also, Chadwick JK et al reported changes in CAL and bone fill of periodontal intrabony defects treated with DFDBA compared to PRF in humans. The result was a significant gain in CAL as well as bone fill after 6 months, with no significant difference between materials.[49]

The mean reduction in defect volume was 38.54±2.17 after 6 months of surgery in Group A. Similarly, in Group B the reduction in defect depth was 49.55±8.56 after 6 months. Both the groups showed a significant reduction in defect volume from baseline. Also, there was statistically significant reduction in defect volume measurements in Group B when compared to Group A (p=0.001*).

The mean increase in Radiographic defect angle was 4.55±0.57 after 6 months of surgery in Group A. Similarly, in Group B the increase in radiographic defect angle was 19.71±0.97 after 6 months. Both the groups showed a significant increase in radiographic defect angle from baseline. Also, there was statistically significant increase in RDA measurements in Group B when compared to Group A (p=0.001*).

In line with the findings of Markou et al and Khosropanah et al, our study revealed an increase in defect angle in both groups. This can be explained by the fact that osseous regeneration starts from the apical region of the root irrespective of the width of the defects, so the newly formed angle would be larger than the baseline angle.[45]

The data interpretation of Radiographic evaluation suggests a significant gain in bone fill in the intrabony defects. These results are in accordance with many studies discussed below.

Kökdere NN et al showed that either PRF used alone or used in conjunction with autogenous bone graft, accelerated the healing of the bone defects. There were statistically significant differences in osteoblast and new bone area values in PRF alone and autogenous graft with PRF than the other groups.[87] So, the reasons for better results in Group B of our study can be due to the additive osteoinductive as well as osteoconductive potential of PRF.

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Simonpieri et al confirmed the validate usage of PRF membranes in reconstruction protocols with FDBA . PRF membrane promotes the soft tissue healing, protects surgical site, and when mixed with graft material forms "biological connector" between the different elements of graft and acts as a matrix which supports neoangiogenesis, capture of stem cells, and migration of osteoprogenitor cells to the center of the graft.[95]

While PRF shows promising results as a graft material for treatment of Intrabony defects, future research is needed, including histologic evaluation of defects treated with PRF to determine if periodontal regeneration is obtained.

A limitation of the present study is the 6-month follow-up time, which could be regarded as rather short, especially for the evaluation of osseous changes. A longer follow-up period might have revealed statistical significance.

CONCLUSION

The conclusions drawn from the study were:

- **Group A :**

- There was a reduction by 1.92±0.01 after 3 months and 3.56±0.02 after 6 months from baseline in PPD and a gain of 1.61±0.01 after 3 months and 1.68±0.02 after 6 months in CAL.
- Defect depth reduced by 1.96±0.57 after 6 months.
- Volumetric bone gain of 38.54±2.17 was observed after 6 months.
- Radiographic defect angle increased by a value of 4.55±0.57 after 6 months.

- **Group B :**

- There was a reduction by 1.92±0.01 after 3 months and 3.56±0.02 after 6 months from baseline in PPD and a gain of 1.32±0.07 after 3 months and 2.30±0.08 after 6 months in CAL.
- Defect depth reduced by 3.27±0.97 after 6 months.
- Volumetric bone gain of 49.55±8.56 was observed after 6 months.
- Radiographic defect angle increased by a value of 19.71±0.97 after 6 months.

On comparison between the two groups Group B (DFDBA+PRF) showed better PI, GI, PPD and CAL results when compared with DFDBA alone group. However, the difference was not statistically significant.

When changes in bone were observed, it was seen that the allograft alongwith PRF group that is Group B showed appreciable changes in radiographic parameters that is there was decrease in defect depth and increase in bone volume and radiographic defect angle. All these were statistically significant when compared with only DFDBA group (Group A).