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Efficacy of Platelet Rich Fibrin as a graft and membrane Vs Hyaluronic acid and Amniotic membrane in the treatment of Grade II mandibular furcation defects: A Randomized Clinical Trial

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Abstract

Context: Furcations of multirooted teeth are very challenging to treat, as their anatomy is complex. Mandibular furcations are more conducive to treatment. Previous studies validated employing graft and membrane to be beneficial in promoting regeneration and defect fill. Platelet rich fibrin (PRF) as a graft and membrane has been employed in many studies and has been successful, however very limited literature is available using hyaluronic acid gel as a graft and Amniotic membrane to treat them.

Aims: This study was done to assess the regenerative potential of 0.2% Hyaluronic acid gel (Gengigel) as a graft material and Amniotic membrane, in comparison to PRF in the treatment of grade II mandibular furcation defects.

Settings and Design: This randomized interventional study included 16 patients aged between 30-50years and was carried out in the outpatient ward of a hospital in Hyderabad. Written consent was taken and the study was registered. (NCT03578744).

Materials and methods: Sixteen Patients with 30 grade II mandibular defects were equally divided into two groups. 8 patients with 15 defects in Group A received PRF, whereas 8 patients with 15 defects in Group B received Gengigel as a graft, over which amniotic membrane was placed. Clinical parameters and amount of bone fill using cone beam computed tomography (CBCT), were estimated at baseline and nine months postoperatively.

Statistical analysis used: The values within the group was done by Paired t test and between the groups by independent sample t test.

Results: Patients in Group B who were administered gengigel as a graft, and amniotic membrane performed better after 9 months pertaining to the reduction in probing depth and bone fill when compared to Group A.

Conclusions: Gengigel when placed as a graft and Amniotic membrane, proved to have better clinical and radiological outcomes when compared to PRF.

Keywords: Grade II mandibular furcation defects, PRF, Gengigel, Amniotic membrane, CBCT.

Key Messages: Furcation defects have been successfully treated with PRF when used as a graft and membrane, however newer materials like hyaluronic acid and amniotic membrane have proved beneficial in treating them.

Introduction

Furcation involvement is a common problem encountered in patients with periodontal disease. As the inter radicular area is affected, treatment strategies should be planned properly and it is mandatory that Grade II defects have to be treated surgically. Previous studies have shown optimal defect fill in Grade II mandibular furcation, when treated with graft and membrane. Different graft materials like autografts, allografts, xenografts and alloplasts have been used along with Guided tissue Regeneration membranes to treat them. However, of late biomimetic materials are administered to promote healing and regeneration of furcation defects. [1]

PRF is a latticework within which cytokines and glycoproteins are enmeshed. PRF as a graft, functions as a skeletal construct for the growth of periosteal cells which favour bone repair. The reason for using PRF as a membrane is due to its potential of protecting the surgical wound and enhancing tissue repair. The membrane connects to the graft and promotes angiogenesis and conducts bone forming cells to the graft. [2]

Gengigel (Ricerfarma, Milano, Italy) has a high percentage of hyaluronic acid and is available as a 0.2% gel for application in periodontal defects. As hyaluronic acid is beneficial for regeneration it has been utilised as a graft material in this study. [3]

The amniotic membrane sticks to the surgical site when placed and therefore microbial contamination is prevented. It contains pluripotent cellular elements which have the potential for transformation into other cells of the periodontium thus making it an ideal choice as a GTR membrane. As this membrane is enriched with stem cells it minimises postoperative scarring and also enhances healing. [4]

As previous studies have shown good results with PRF when used as a graft and membrane in grade II mandibular furcation defects, this study aimed to assess its regenerative potential in comparison to gengigel and amniotic membrane.

Text

Subjects and Methods: Sixteen patients with Grade II furcation involvement of the lower arch participated in this study which was done from November 2017 to August 2018 in an outpatient ward of a hospital in Hyderabad. The criteria related to the Helsinki declaration of 1975 as revised in 2000 were adhered to and the institutional review board approved of the study.

Statistical Package for Social Sciences (SPSS) version 22.0 (Armonk NY: IBM United States. IBM Corp. 2013 was used for the statistical analysis. The tests were performed at 95% confidence interval and $P<0.05$ was considered to be statistically significant. Differences within the groups from baseline to 9 months postop was computed using paired t test and between the groups was analysed using independent t test.

Selection Criteria: Patients with Probing depth $\geq$ 5mm and Relative vertical clinical attachment level (RVCAL) and relative horizontal clinical attachment level (RHCAL) $\geq$ 3mm participated. Non-compliant patients, smokers and systemically compromised individuals, patients taking medications which are known to interfere with periodontal wound healing; pregnant or lactating women; In addition, teeth with mobility >Grade II were excluded.

The samples were screened and randomly assigned in sealed envelopes by investigator KRR into PRF (Group A) and Gengigel +Amniotic membrane (Group B). The treatment was performed by investigator LB. (Fig 1)

Clinical assessment for Plaque index(PI), Pocket depth(PD), RVCAL were made using Williams probe. The RHCAL was assessed with a Naber's probe. Cone beam computerised tomography(CBCT) was used pre and postoperatively to assess the amount of defect fill in the furcation of the affected teeth.

An Occlusal stent was prepared on the model which was obtained by taking impressions of the mandibular arch of all the patients participating in the study. The RVCAL was determined by calculating the distance from the reference point (lower border of stent) to the base of the pocket which was subtracted from the distance from the lower margin of stent to the cemento enamel junction. The RHCAL was measured with a Naber's probe from the lower margin of the stent into the furcation fornix

Standardization of radiographic technique: All the CBCT scans were taken by the same person pre and post-surgery. The sagittal and coronal sections were reconstructed after 9 months at the same axial slicing to that of the baseline. The CBCT measurements were done both presurgically and 9 months after surgery. (Fig 2 and Fig 3)

Outcome measures: Primary measure assessed was the amount of defect fill in the furcation, whereas the secondary measures were the PD, RVCAL and RHCAL.

Pre-Surgical Procedure The participants underwent scaling and root planing and were given instructions on both mechanical and chemical plaque control.

Surgical Procedure The patients were examined 8 weeks after the phase I therapy to finalise their participation in the surgical protocol The site to undergo surgery was anaesthetised using local anaesthetic. A no 15 Bard parker blade was used to give the crevicular and interdental incision. The muco-periosteal flap was then reflected with the help of a periosteal elevator, and the necrotic tissue was debrided from the defect site using furcation curettes. The sites were then thoroughly irrigated. PRF as a graft and membrane was placed in the furcation defects of patients belonging to Group A. (Fig 4)

Protocol for PRF procurement: 10 ml of blood from antecubital vein was drawn, and then transferred into empty sterile glass test tubes. Then the sample underwent centrifugation at 3000 rpm for 10 minutes in a Remi centrifuge. The clot procured after centrifugation was compressed between sterile gauze pieces and was then shaped to be used as a graft and a membrane.

Group B sites after flap elevation and debridement of the defect received 0.2% hyaluronic acid (Gengigel) as a graft. Then treated amniotic membrane (procured from Tata memorial hospital, Mumbai) was shaped and placed over the graft and stabilised. (Fig 5) The flap was then sutured back into position using 3.0 black silk suture. The Coe pack dressing was given and patients were recalled after two weeks.

Postoperative Care: Instructions were given to all patients after surgery and they were administered antibiotic and analgesics. (Amoxycillin 500 mg thrice a day for five days and Acecloren containing 100mg aceclofenac,325mg paracetamol and 15mg seratiopeptidase, three times a day for four days). Dressing and sutures were removed after 15 days. Patients were motivated for oral hygiene care, and were recalled for follow ups, weekly for the 1st month postop and subsequently after 3 months and 9 months respectively. The clinical parameters were reassessed at 3 months and 9 months whereas the radiological parameters (CBCT) were assessed after 9 months.

Results: On intragroup comparison of all the variables, it was observed that there was a marked difference pertaining to all of them from baseline to 9 months postop.

(P<0.001) (Table 1). However, when an intergroup comparison was made it was seen that there was a noticeable improvement only in the pocket depths (PD)and depth of defect fill (DPF) in Group B over Group A 9 months' post -surgery. The PD in Group A was 5.80mm at baseline and it reduced to 1.93, whereas in Group B it was 6.20mm at baseline and reduced to 1.47mm (P<0.03) The DPF was 3.59mm at baseline in Group A which reduced to 1.54mm 9 months postop (Fig 6) and was 3.91mm at baseline in Group B which improved to 1.25mm (Fig 7) after 9 months (P<0.01) (Table2).

Discussion

The posterior teeth are easily affected by periodontitis as they are multi rooted and have complex anatomical features. Attachment loss progresses rapidly in the posterior dentition causing furcation involvement. Access to the furcation is hampered by its shape and size not being amenable for treatment with normal Gracey curettes. Therefore

specialized furcation curettes have been developed and used to treat them. [5]

The maxillary molars are affected more with furcation involvement when compared to the mandibular. 53% of the maxillary first molars have been studied to be affected with distal furcation involvement. [6] Clinically the furcation involvement has been diagnosed using Nabers probe for horizontal clinical attachment levels, University of North Carolina 15 probe (UNC 15) and Williams probe for vertical clinical attachment levels. Pertaining to the radiological measurement of the furcation defect, CBCT is now considered the most reliable tool. [7-8]

The use of CBCT was advocated in this study to measure the height, width and depth of the furcation defects. Regeneration of periodontal tissues has been attempted by clinicians for quite some time. In relation to furcation defects many graft materials and guided tissue regeneration membranes (GTR)have been employed with moderate success, however of late biomimetic agents like PRF has been tried in furcation defects. PRF plays an important part in tissue regeneration due to its inherent property of growth factor release, from one week to 28 days' post application. Moreover, proteolysis of growth factors does not occur when PRF is used as a therapeutic agent, because it is a natural fibrin meshwork without thrombin. [9]

Some researchers stated that PRF as a GTR membrane, creates adequate space facilitating cellular events that are conducive for periodontal regeneration leading to bone formation. [10]

Other researchers who studied the effect of PRF on human periodontal ligament fibroblasts and its use in periodontal infrabony defects observed that it was found to increase protein kinase phosphorylation and osteoprotegerin in periodontal ligament fibroblasts, and also found to upregulate alkaline phosphatase activity. There was a reduction in the pocket depth and a CAL gain with defect fill 6 months postoperatively. [11]

In another study 18 patients with 36 mandibular degrees II furcation defects were randomly allotted and treated either with OFD and autologous PRF as a graft and membrane (test group) or OFD alone (control group). PI, SBI, PD, RVCAL, RHCAL, GML, and radiographic investigations were performed at baseline and 9 months postoperatively. It was observed after 9 months that there was a marked improvement in the PD in the test group than the control group with a difference of 2.17mm. The vertical defect fill was also greater in the test group (50.8 ±6.24) when compared to the control group (16.7 ± 6.42) at nine months [12]

Yet another study evaluated 22 patients with grade II mandibular furcation defects who were equally divided

into two groups. PRF as a graft and as a membrane was placed in patients belonging to Group A, whereas, allograft and healiguide collagen membrane were placed in the defects of patients categorized under Group B. PI, PD, RVCAL, RHCAL, Gingival Marginal Level(GML), and amount of Bone fill using Radio-Visiography (RVGBF), were estimated at baseline and nine months postoperatively. Though there was no improvement in the clinical parameters between the groups after 9 months, there was a measurable improvement in the RVCAL (p-value=0.04 Sig) and RVGBF (p-value=0.006 Sig) in relation to Group A when compared to Group B. [2]

This study also employed PRF as a graft and also as a membrane, which might have contributed to the improvement in clinical and radiographic parameters in Group A (Table 1)

The periodontal ligament matrix comprises of hyaluronic acid which participates in cell adhesion, migration and differentiation mediated by various HA binding proteins and cell surface receptors such as CD44. The periodontal tissues express CD44 antigen and it has been observed that when hyaluronic acid interacts with CD44, there is increased cellular proliferation and mineralization within the periodontal ligament. [13]

HA due to its it's anti-inflammatory activity promotes the healing in soft and hard tissues which may be of significant interest during periodontal regeneration. Many studies have reported the beneficial effects of HA pertaining to a reduction in the bleeding on probing as well as the probing pocket depths. It was observed that PDL cell viability increased when HA was administered leading faster hard tissue differentiation. [14]

Some researchers who conducted an animal study opined that hyaluronan is the best carrier for the bone morphogenic proteins (BMP), the growth factors commonly documented to stimulate the formation of novo bone. [15]

Twelve patients with chronic periodontitis were recruited in another study which estimated the adjunctive effect of 0.8% Hyaluronan as a gel to SRP, in the treatment of chronic periodontitis The group administered HA+SRP showed a marked reduction in the bleeding on probing as well as the probing pocket depths versus SRP alone. [16]

In another study, the effect of HA gel (0.8%}, in conjunction with periodontal surgery was evaluated in fourteen patients with chronic periodontitis. The inclusion criteria were four interproximal intra bony defects (≥3 mm) with probing depth >5 mm. After phase 1 therapy, defects were randomly assigned to modified Widman flap surgery in conjunction with HA gel (test) or placebo gel (control) application. Measurable differences were noted for CAL and gingival recession, ($P < 0.05$) in favour of the test sites. But other clinical parameters were not significant on intergroup comparison. [17]

In another study a total of 20 sites from 10 patients, with Grade II furcation defects were selected using random sampling technique. The patients in Group A were treated with placement of HA gel as a graft and coronally displaced flap, whereas in Group B, patients were treated with coronally displaced flap only, after furcation debridement in both the groups. Furcation defect assessment was done preoperatively and postoperatively at six months through surgical re-entry. They concluded that the combination of HA gel with coronally positioned flap leads to better results as compared to coronally positioned flap alone. [18]

Periodontal regeneration has been observed to be enhanced by the amniotic membrane (AM), lining the placenta comprised of a single layer of epithelium followed by a thick basement membrane with an avascular stroma. It has a wide array of uses which include as a local

drug delivery agent, as a graft material after vestibuloplasty, as a GTR membrane in treatment of periodontal osseous defects, gingival recessions and furcation defects. [19]

The epithelial layer of the amniotic membrane retains a reservoir of stem cells which creates a natural scaffold for self-seeding in tissue engineering. Amniotic membrane can modulate angiogenesis and promote wound healing. The wound healing property is further enhanced by the physiological seal obtained with gingiva. [20]

Fifteen patients with thirty mandibular grade II furcation defects, formed the samples for another study wherein they were treated with PRF and AM membrane (Group I) or PRF only (Group II). Clinical parameters were recorded at baseline, 3 months, and 6 months postoperatively. Radiographic assessment was done at baseline and 6 months postoperatively aided by computerised axial tomography (Dentascan). All clinical and radiographic parameters showed statistically significant improvement at the sites treated with PRF and amnion membrane compared to those with PRF alone. [21]

Ten patients having similar intra bony defects (IBD) in a split mouth design, were randomly treated with both open flap debridement (OFD) and decalcified bone allograft (DFDBA) (Control group) and OFD, DFDBA and AM (Test group}. Clinical and radiographic variables were examined. In this study both the groups showed similar improvement in all the parameters assessed. [22]

Another study included a patient with a grade II furcation in the lower right mandibular first molar. Hyaluronic acid gel (Gengigel) along with bioactive amnion membrane was placed in the furcation area during the surgical phase. The defect was assessed radiographically at 4 months and 6 months postoperatively. It was inferred that surgical placement of Gengigel along with amnion membrane in the furcation defect can significantly improve the periodontal defect morphology. [23]

In the present study also it was observed that there was a marked improvement in the clinical parameters as well as the defect fill in Group B (Table 1). However, when an intergroup comparison was made it was seen that there was a marked improvement only in the pocket depths (PD) and depth of defect fill (DPF) in Group B over Group A 9 month's post –surgery. (Table 2)

Limitations of the study: This study was not a split mouth design and follow up period in this study was 9 months only. More research with an extensive study period and larger sample size is required to validate the results.

Conclusion: Both Group A (PRF as a graft and membrane) and Group B (Gengigel and Amniotic membrane) were biocompatible with the tissues and showed improvement in all the parameters at 9 months. A significant improvement was only noticed pertaining to the PD and the depth of defect fill in Group B over Group A. More research with an extensive study period and larger sample size is required to confirm the results obtained.

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Legend Tables

Group	Variables	Base line		9Months Post Surgery		Difference		P Value
		Mean	SD	Mean	SD	Mean	SD	
A	PI	1.83	0.98	0.39	0.22	1.44	1.03	<0.01 Sig*
	PD	5.80	0.77	1.93	0.59	3.87	0.64	<0.01 Sig*
	RVCAL	4.27	0.80	0.60	0.83	3.67	0.62	<0.01 Sig*
	RHCAL	2.93	0.80	0.27	0.59	2.67	0.72	<0.01 Sig*
	HTF	2.44	0.66	0.87	0.30	1.57	0.52	<0.01 Sig*
	WDF	2.20	0.49	0.87	0.27	1.34	0.36	<0.01 Sig*
	DPF	3.59	0.42	1.54	0.30	2.05	0.37	<0.01 Sig*
B	PI	1.99	0.51	0.53	0.31	1.45	0.34	<0.01 Sig*
	PD	6.20	0.68	1.47	0.52	4.73	0.46	<0.01 Sig*
	RVCAL	4.73	0.70	0.33	0.62	4.40	0.51	<0.01 Sig*
	RHCAL	3.27	1.03	0.33	0.62	2.93	0.70	<0.01 Sig*
	HTF	2.72	0.60	0.81	0.35	1.91	0.40	<0.01 Sig*
	WDF	2.37	0.52	0.76	0.31	1.61	0.35	<0.01 Sig*
	DPF	3.91	0.61	1.25	0.25	2.66	0.62	<0.01 Sig*

Table 1: Intragroup Comparison of Outcome measures.

PI-Plaque index, PD- Probing Depth, RVCAL-Relative vertical clinical attachment level, RHCAL-Relative horizontal clinical attachment level, HTF-Height of furcation, WDF-Width of furcation, DPF-Depth of furcation, SD-Standard deviation, P value -Probability value, <0.01Sig*-Statistically significant.

Variables	Groups				P_Value	Significance
	PRF as graft & membrane (GROUP A)		Amniotic membrane and Gengigel (GROUP B)			
	Mean	SD	Mean	SD		
PI Baseline	1.83	0.98	1.99	0.51	0.60	NS
PI_9months	0.39	0.22	0.53	0.31	0.17	NS
PD Baseline	5.80	0.77	6.20	0.68	0.14	NS
PD 9months	1.93	0.59	1.47	0.52	0.03*	SIG
RVCAL Baseline	4.27	0.80	4.73	0.70	0.10	NS
RVCAL 9months	0.60	0.83	0.33	0.62	0.33	NS
RHCAL Baseline	2.93	0.80	3.27	1.03	0.33	NS
RHCAL 9months	0.27	0.59	0.33	0.62	0.77	NS
HTF_Baseline	2.44	0.66	2.72	0.60	0.23	NS
HTF 9months	0.87	0.30	0.81	0.35	0.62	NS
WDF Baseline	2.20	0.49	2.37	0.52	0.37	NS
WDF 9months	0.87	0.27	0.76	0.31	0.32	NS
DPF Baseline	3.59	0.42	3.91	0.61	0.11	NS
DPF_9months	1.54	0.30	1.25	0.25	0.01*	SIG

Table 2: Intergroup comparison of outcome measures.

PI-Plaque index, PD- Probing Depth, RVCAL-Relative vertical clinical attachment level, RHCAL-Relative horizontal clinical attachment level, HTF-Height of furcation, WDF-Width of furcation, DPF-Depth of furcation, SD-Standard deviation, P value- Probability value, 0.03* and 0.01*-Significant, NS-Not significant, SIG-Significant.

Legend Figure

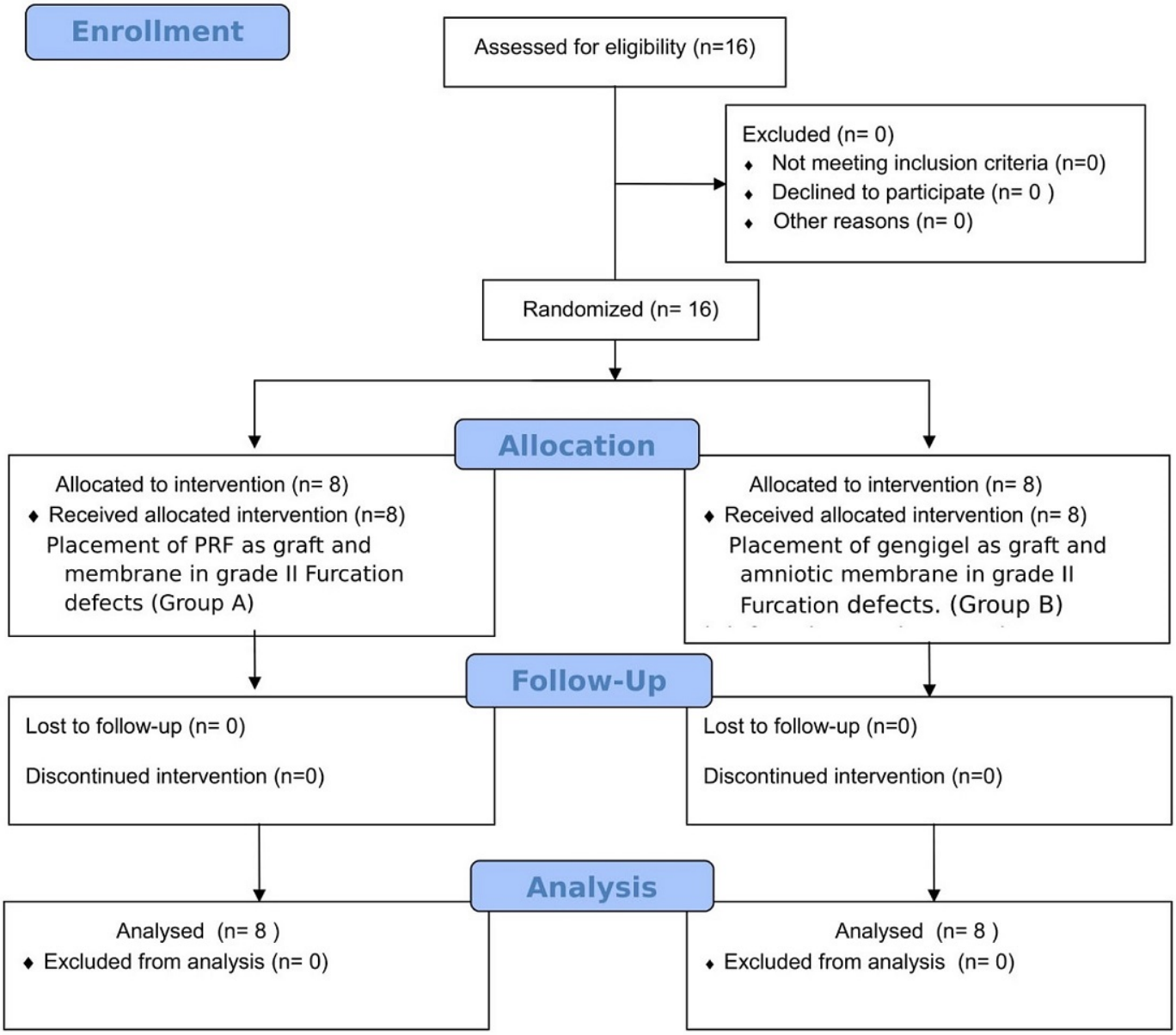

Fig 1: CONSORT Flow diagram

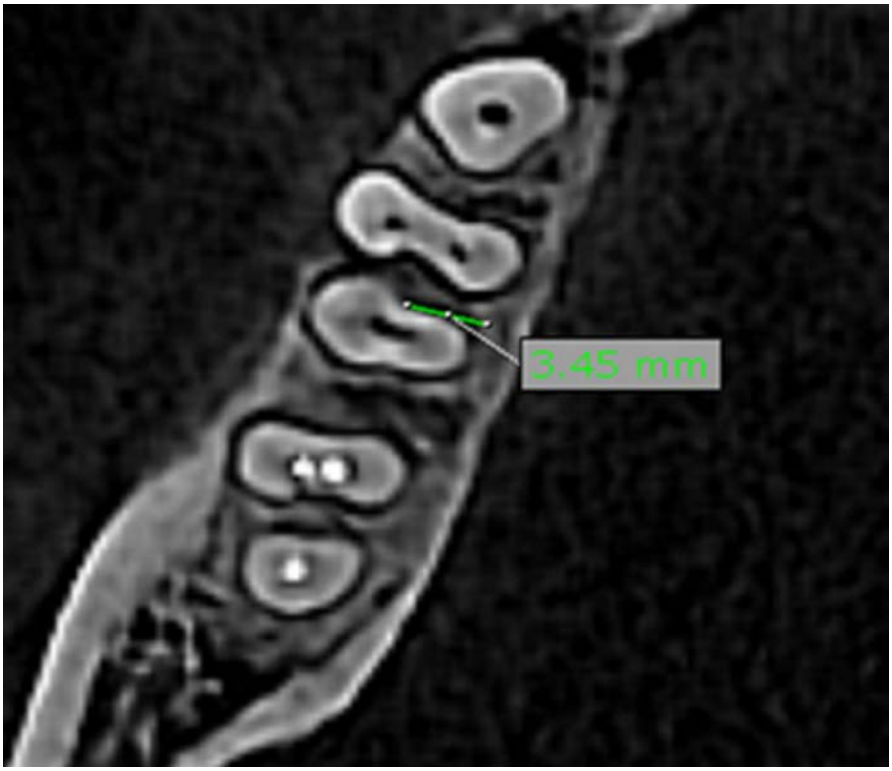

Fig 2: Preop depth of furcation in PRF group

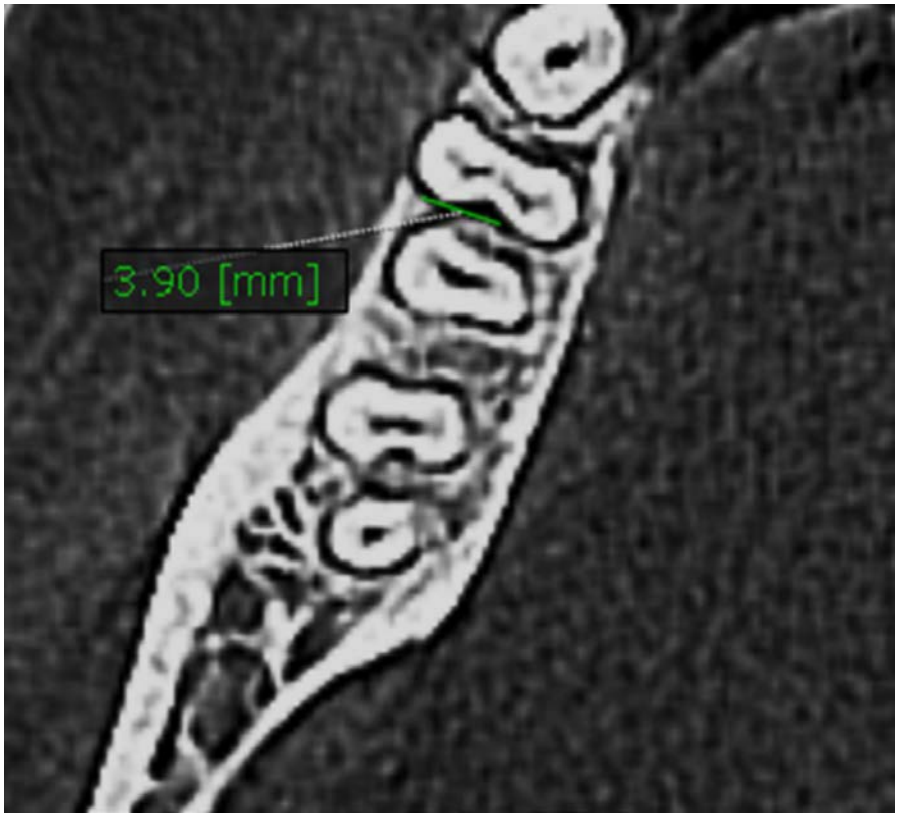

Fig 3: Pre-op depth of furcation in Amniotic membrane group

Fig 4: Placement of PRF as a graft and membrane

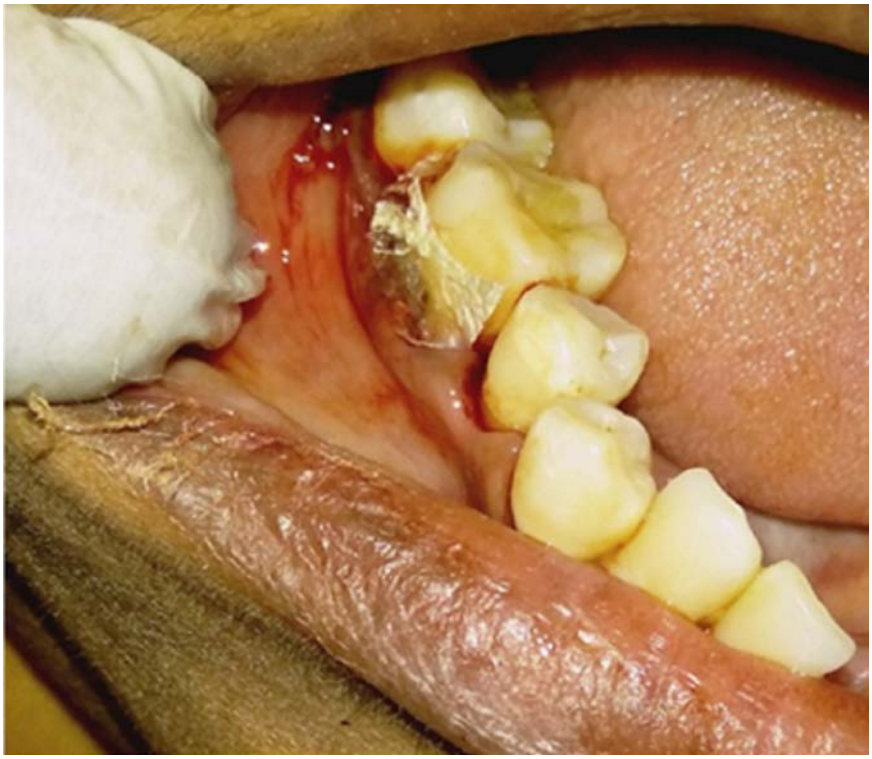

Fig 5: Placement of Amniotic membrane

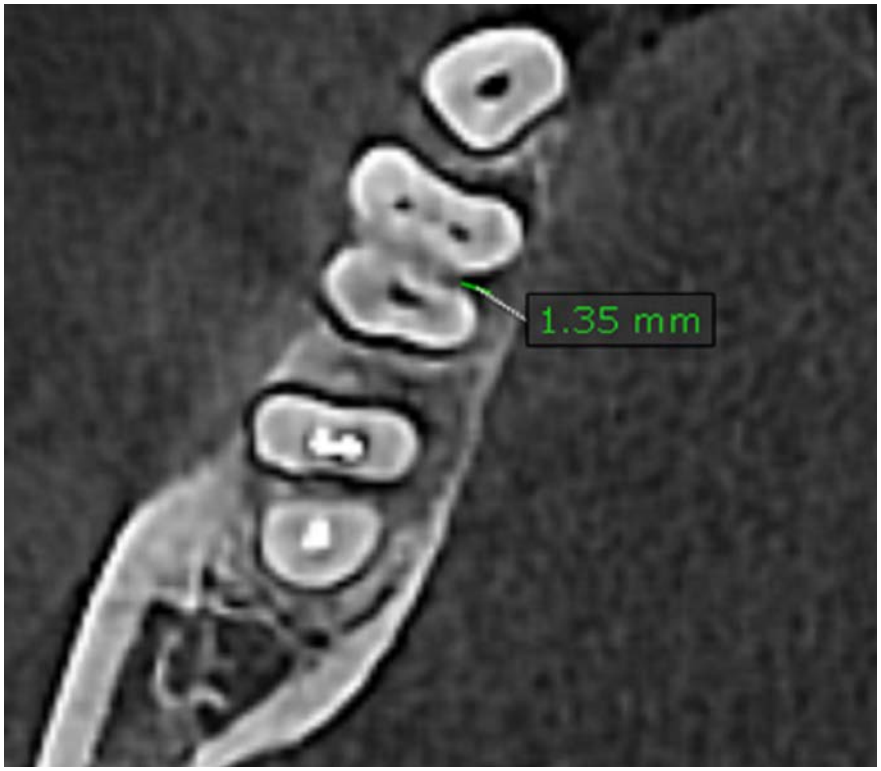

Fig 6: Postop depth of furcation in PRF group

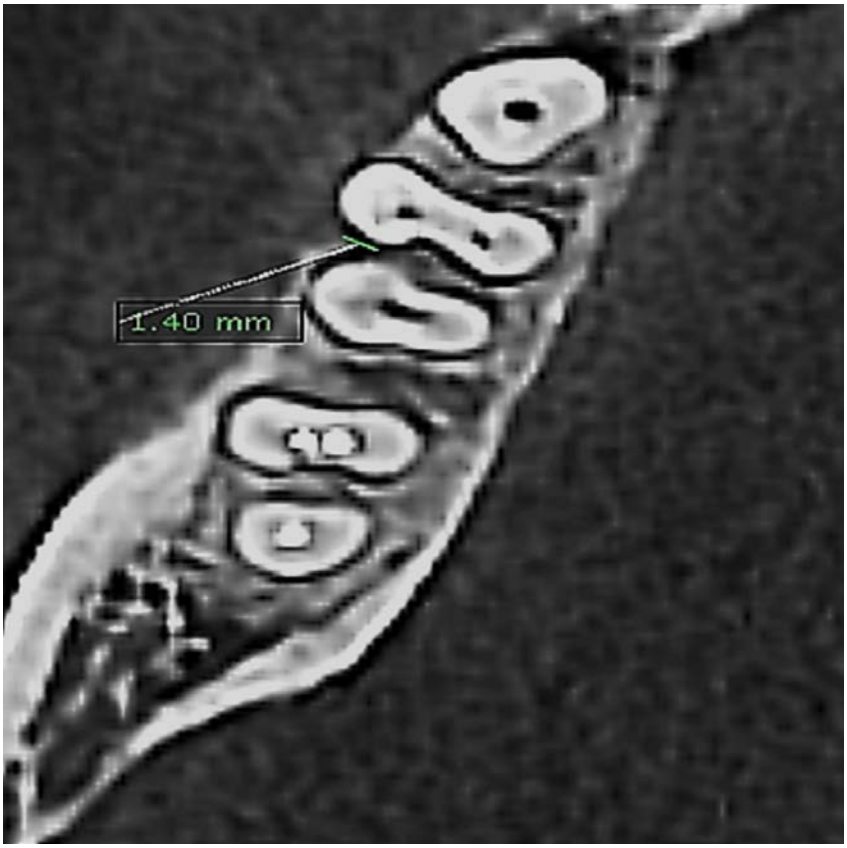

Fig 7: Postop depth of furcation in Amniotic membrane group.