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RESEARCH ARTICLE
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COMPARATIVE EVALUATION OF HUMAN PERIODONTAL OSSEOUS DEFECTS TREATED WITH AUTOGENOUS BONE GRAFTS AND LYOPHILISED IRRADIATED AMNIOTIC MEMBRANE: A CLINICO-RADIOGRAPHIC STUDY

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Abstract

Background and Aims: Periodontal regeneration is a complicated process involving a number of different cell types and cell-stromal interaction resulting from the ability of cementoblasts, periodontal ligament cells, and osteoblasts to form a new periodontium. Improved understanding of the ability of various growth factors to regulate these cell types may lead to significant improvements in our ability to regenerate the periodontium. Among the graft materials to date, only autogenous bone of extra-oral and intra-oral sources is considered as the 'gold standard' because it provides the three elements required for bone regeneration – osteogenesis, osteoconduction and osteoinduction. The amniotic membrane has gained importance specifically because of various factors. The purpose of this study was to evaluate the efficacy of Autogenous graft and lyophilised irradiated Amniotic membrane versus Autogenous graft in the treatment of intrabony defects.

Material and Methods: Twenty-four intra-bony defects (15 patients) following open flap debridement were treated by Autogenous graft and lyophilised irradiated Amniotic membrane or Autogenous graft alone. Clinical parameters such as plaque index (PI), gingival index (GI), gingival bleeding Index (GBI), probing pocket depth (PPD), clinical attachment level (CAL) and gingival marginal position (GMP) were recorded at baseline, 3 months, 6 months and 9 months post-operatively. In both the groups radiographic assessment was done for each site at baseline, 3 months, 6 months and 9 months post-operatively using IOPA paralleling technique and analyzed using *COREL DRAW 7* software.

Results: The results showed significant reduction of clinical parameters (PI, GI, GBI, PPD) and radiographic parameters (amount and percentage of bone fill) and significant gain in CAL in both treatment groups which were statistically non-significant on inter group comparison. The mean

pocket reduction was statistically significant at baseline and 9 months and non-significant on inter group comparison. The percentage of original defect filled, and percentage of original defect resolved at baseline and 9 months were statistically not significant, whereas the percentage change in alveolar crest was highly significant.

Interpretation and Conclusion: Within the limits of the present study, there was greater reduction in PPD, more CAL gain and greater intra-bony defect fill at sites treated with Autogenous graft and lyophilised irradiated Amniotic membrane than with Autogenous graft alone. Thus, Autogenous graft and lyophilised irradiated Amniotic membrane can be considered as an effective alternative for the treatment of periodontal osseous defects.

Key words: Lyophilised irradiated Amniotic membrane, Autogenous graft, Intrabony defect, Periodontal regeneration, Open flap debridement.

INTRODUCTION

Periodontal therapy has an ultimate goal of predictable regeneration of the periodontium at the site of previous marginal periodontitis. A major factor inhibiting predictable regeneration appears to be the nature of periodontitis affected root surface. The exposed root surface associated with periodontitis undergoes substantial alterations. The fiber attachment system is destroyed, resulting in denuded and contaminated root surface. Various periodontal treatment modalities are attempted to obtain the lost periodontal structures.

Currently, the most often used regenerative techniques involve the use of bone inductive graft materials, guided cell repopulation using barrier membranes and coronally repositioned flaps procedures in which the flap margin is secured at an appreciable distance from the healing site[1]All the 3 techniques demonstrated clinical success, in varying degrees in promoting periodontal regeneration and /or bone fill in periodontally diseased sites.

During the 1989 world workshop in clinical periodontics, the concern was that the research should be directed at combination treatments for periodontal regeneration, involving bone grafts in conjunction with barrier membranes. Since then, several human studies have been undertaken, with most involving GTR with autografts[2]. Bone grafts and their synthetic substitutes have been used in an attempt as a regenerative modality. Among the graft materials to date, only autogenous bone of extra and intra oral sources is considered as the 'gold standard' because it provides the three elements which are required for bone regeneration- osteogenesis, osteoconduction and osteoinduction[3].

The scanty literature and lack of comparative studies on periodontal regeneration by Autogenous bone graft and lyophilised irradiated Amniotic membrane opens a new avenue for such a study. Hence the present study has been undertaken to evaluate the efficacy of Autogenous bone graft and lyophilised irradiated Amniotic membrane in the treatment of interproximal vertical bony defects both clinically and radiographically[4,5].

METHODOLOGY

The patients for the study were selected from the outpatient Department of Periodontics, College of Dental Sciences, Davangere, Karnataka. Written informed consent from patients and ethical clearance from the Ethical committee of College Of Dental Sciences,Davangere, Karnataka was obtained. The study period was from December 2015 to August 2017.

INCLUSION CRITERIA:

Patients of both the sexes with age group between 20-45 years having at least one intrabony defect with a probing depth of 5mm, radiographic evidence of vertical bone loss and presence of inter-

proximal vertical defects ≥3mm deep (distance between alveolar crest and base of the defect) on radiograph.

EXCLUSION CRITERIA
Patients on antibiotics one month prior to the study, poor oral hygiene during pre-surgical period, history of systemic diseases, patients who had undergone any type of periodontal therapy 6 months, use of tobacco or tobacco related products, allergic to drugs, pregnant or lactating women, patients taking any medication that may interfere with wound healing.

STUDY DESIGN:
A randomized case-controlled trial, total of 24 sites in 15 patients (11 females, 4 males) with moderate periodontitis were included in this parallel design study

Control Group:12 sites were treated with open flap debridement followed by placement of Autogenous bone graft.

Experimental Group: 12 sites were treated with open flap debridement followed by placement of Autogenous bone graft and Lyophilised Irradiated Amniotic membrane.

CLINICAL ASSESSMENT:
The following clinical parameters were recorded at baseline, 3, 6 and 9 months post-surgery.
1. Plaque Index (PI) (Silness P. and Loe H., 1964)
2. Gingival Index (GI) (Loe H. and Silness J, 1963)
3. Gingival Bleeding Index (GBI) (Aianamo and Bay, 1975)
4. Gingival marginal postion (GMP)
5. Probing pocket depth (PPD)
6. Clinical attachment level (CAL)

STENT PREPARATION:
Study models were used for fabrication of customized acrylic occlusal stents. The stent was made using self-cured pink acrylic which covered the occlusal as well as the coronal 1/3rd of the labial or buccal and lingual or palatal surfaces of the tooth involved and one tooth mesial and distal to the involved tooth. Vertical grooves were made to guide the placement of the probe in the same plane and direction repeatedly during measurements to avoid any variation. The lower limit of the vertical groove was used as the fixed reference point (FRP).

CLINICAL MEASUREMENTS RECORDED USING STENT:
1. FRP to base of the pocket (BOP).
2. FRP to cemento enamel junction (CEJ).
3. FRP to gingival margin (GM).

The following calculations were made from the clinical measurements recorded.
i. Pocket depth = (FRP to BOP) – (FRP to GM)
ii. Clinical attachment level = (FRP to BOP) – (FRP to CEJ)
iii. Gingival margin position = (FRP to CEJ) – (FRP to GM)

The recordings were made using ***University of North Carolina*****15** ***probe (Hu Friedy*****)**

RADIOGRAPHIC ASSESSMENT:
Intraoral periapical (IOPA) radiographs of each defect site were exposed at baseline, 3, 6 and 9 months using paralleling technique and subjected for the measurement of osseous defect(Figure 13-16). The following parameters were measured:

1. Amount of defect fill
2. Percentage of fill of original defect

Amniotic membrane graft preparation:
Lyophilised and irradiated Amniotic membrane (3*3cm) was obtained from a commercial tissue bank (Tata Memorial Hospital, Mumbai, India). Amniotic membrane is stable and can be stored at room temperature.

PRE-SURGICAL PROCEDURES:
All the selected patients, following an initial examination and treatment planning discussion were given detailed instructions in self-performed plaque control measures and were subjected to initial periodontal therapy. 2-3 weeks after phase-I therapy the oral hygiene status and the tissue response was evaluated using plaque index, gingival index, gingival bleeding index. If the oral hygiene was acceptable i.e., PI ≤1, patients were subjected to surgical procedure.

SURGICAL PROCEDURE:
On completion of the baseline examination and thorough initial therapy (scaling and root planing), the intrabony defects were randomly assigned to either experimental or control groups. Patients were seated comfortably on the dental chair and then asked to rinse the mouth with 10 ml of 0.2% chlorhexidine digluconate solution. The extraoral surfaces of the patient were swabbed with 0.5% povidone iodine solution. The operative site was anaesthetized with 0.2% Lignocaine HCI with adrenaline (1:80,000) using block and infiltration techniques. The crevicular and interdental incisions were given using the Bard Parker handle with blade No12 and No.15. A full thickness mucoperiosteal flap was reflected using the periosteal elevator. After reflection of the flap and exposure of osseous defect, a thorough surgical debridement of soft and hard tissue was done using area specific Gracey curettes. After completion of debridement the surgical site was irrigated copiously with 0.9% normal saline and carefully inspected to ensure that the procedure had been completed. Pre suturing was done at the experimental group and the control group through the buccal and lingual flap. Before the placement of the lyophilised irradiated Amniotic membrane a sterile template was prepared to measure the defect for the lyophilized irradiated Amniotic membrane to perfectly fit and fill the defect and the lyophilised irradiated Amniotic membrane was sized according to the pre-measured sterile template and subsequently placed into the defect[6-8].

In the control group, the osseous defects were filled with Autograft and in the experimental group the osseous defects were filled with autograft and lyophilised irradiated Amniotic membrane.

Autogenous bone was harvested from the mandibular symphysis region. Assessment of the periodontal status of lower anteriors, amount of bone loss, periodontal risk of root fenestration, amount of keratinized gingiva, subgingival margins of the restoration and local musculature was done to indicate the best possible incision design. The trephine bur used in this study were of 2mm and 4 mm diameter sizes; cores of 4-10 mm were harvested[9,10]. (Figure 1- 13)

The mucoperiosteal flaps were repositioned and secured in place using a 3/8 circle, reverse cutting needle and 3-0 black braided silk sutures. Interrupted direct loop sutures were placed to obtain primary closure of the interdental papilla and the area was protected with a non-eugenol *(Coe-pak)* dressing. All patients were prescribed systemic Amoxicillin 500mg, Metrogyl 400mg thrice daily for 5 days along with Combiflam tablets thrice daily for 3 days and 0.2% chlorhexidine digluconate (CHX) rinse twice daily for 2 weeks. Post-operative instructions were given to all patients and they were instructed to report to the department after 24 hours of surgery and then after 10 days.

SURGICAL PROCEDURE

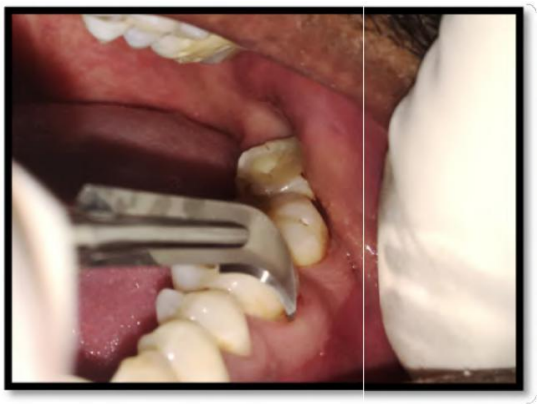

Fig 1: Incision Placement

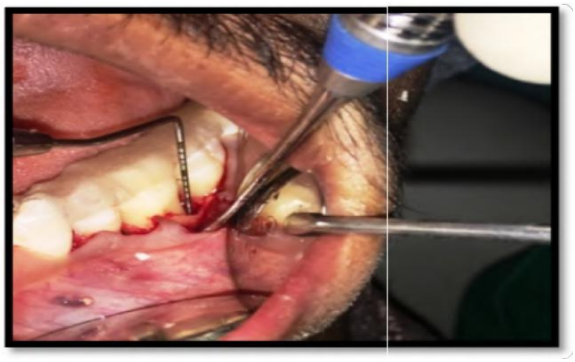

Fig 2:FRP to CEJ

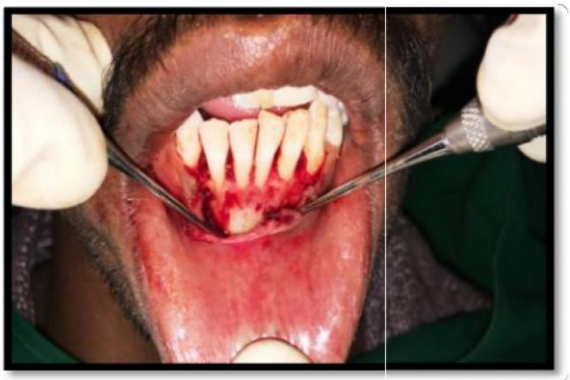

Fig 3: Exposure of donor site (symphysis)

Fig 4: Harvesting of Autograft with Trephine bur

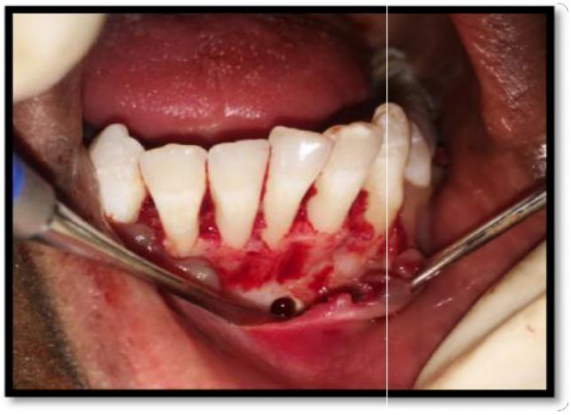

Fig 5: Donor site

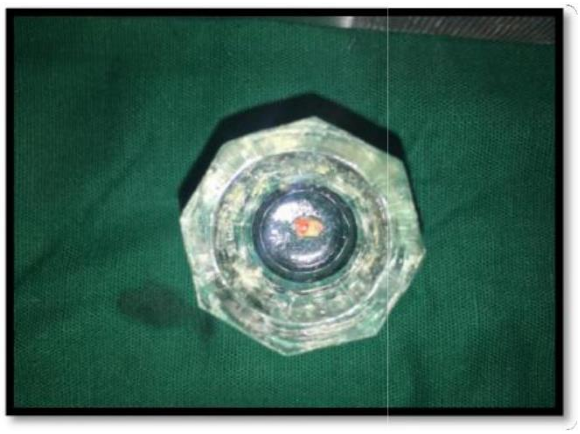

Fig 6: Harvested autogenous bone graft

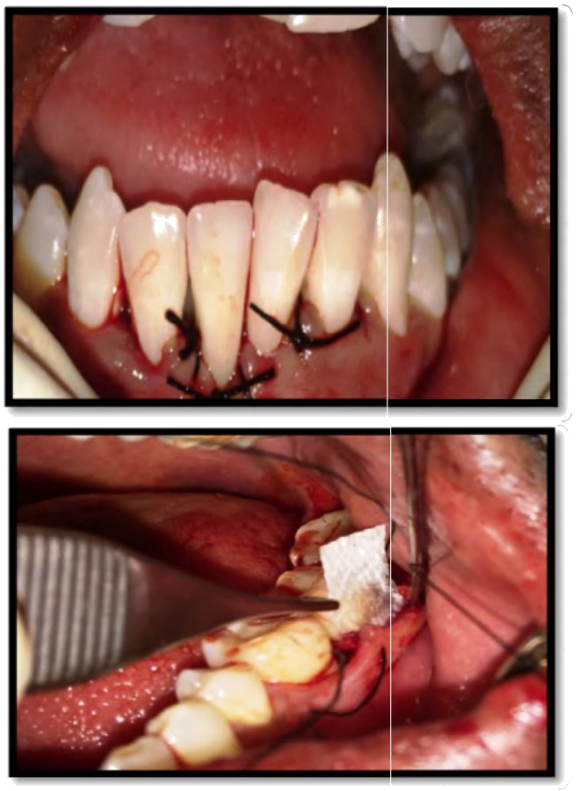

Fig 7: Sutures placed in donor site

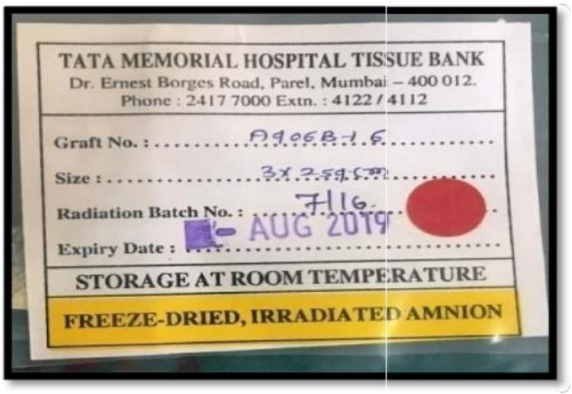

Fig 8: Lyophilised Irradiated Amniotic membrane

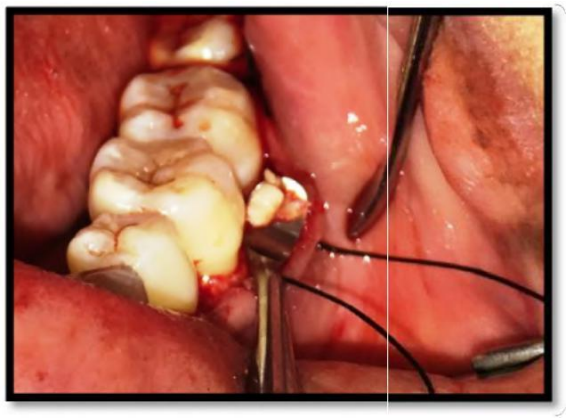

Fig 9: Placement of the Autogenous graft

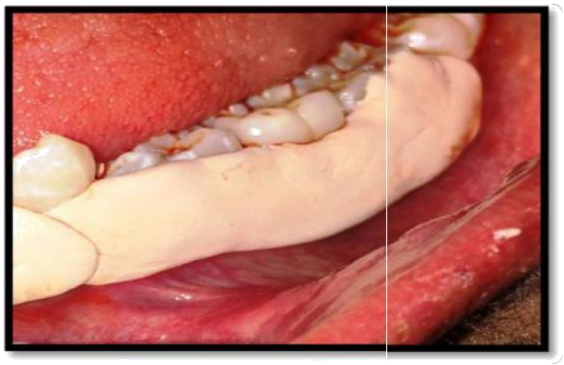

Fig 10: Placement of Amniotic membrane

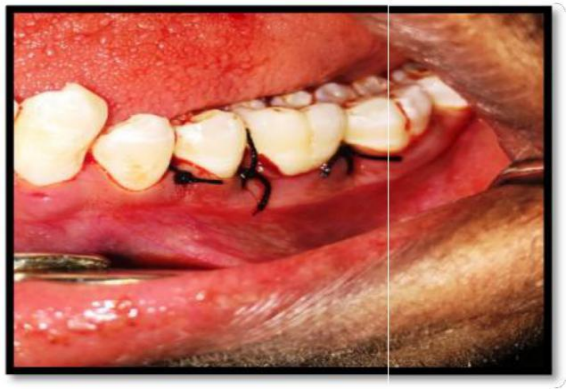

Fig 11: Sutures placed in the recipient site

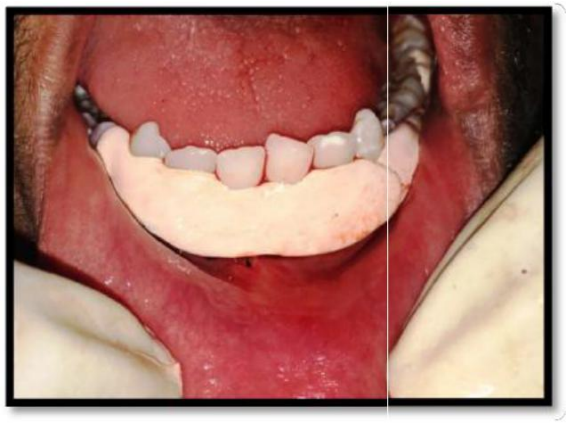

Fig 12: Periodontal pack placed in the donor site

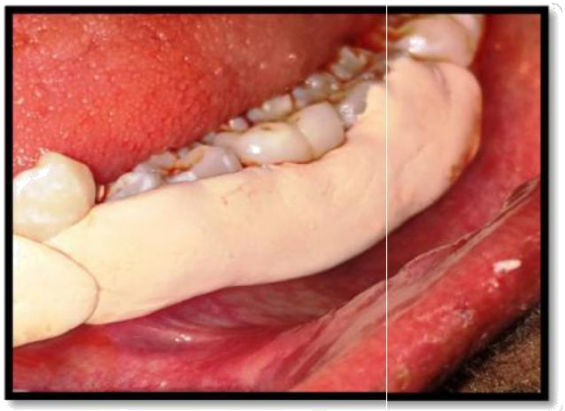

Fig 13: Periodontal pack placed in recipient site

POST OPERATIVE CARE:

Periodontal dressing and sutures were removed after 1 week post operatively. Surgical wounds were gently cleansed with 0.2% CHX on a cotton swab. Thereafter gentle brushing with a soft tooth brush was recommended. At 8 weeks post-operatively, each patient was reinstructed about proper oral hygiene measures. Patients were re-examined weekly for 1 month after surgery and then at 3, 6 and 9 months. Post operative care included reinforcement of oral hygiene and mechanical plaque control whenever necessary. (Figure 14- 16). The results were subjected to statistical analysis.

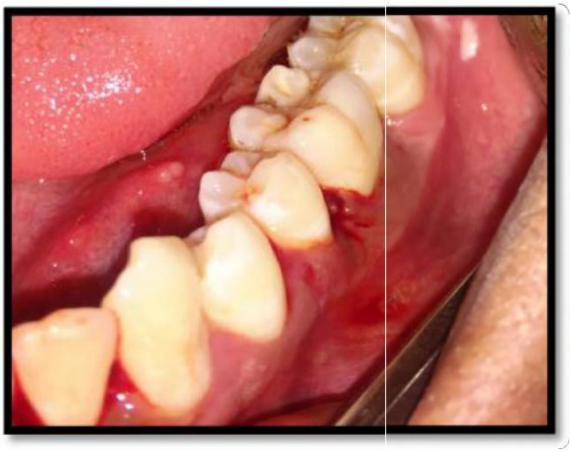

Fig 14: 1 week recipient site post-operative

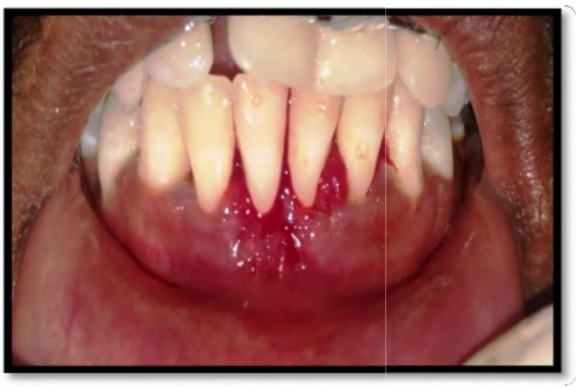

Fig 15: 1 week donor site post-operative

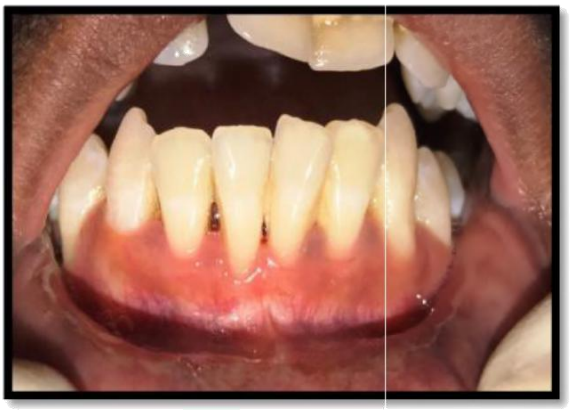

Fig 16: 9 months donor site post-operative

MEASUREMENT OF OSSEOUS DEFECTS:

IOPA of each site were digitalized using a flatbed scanner with a scanning resolution of 600 dpi *(UMAX – ASTRA 1220S).* The scanned images, stored in JPEG format were transferred to *COREL DRAW 7*. Using the ruler tool, a line was drawn from CEJ to the base of the defect. The software then displayed the distance between these two points. The same procedure was then repeated to obtain the distance between CEJ and alveolar crest. Subtracting these two measurements, the depth of osseous defect was obtained.

STATISTICAL ANALYSIS:

Results were expressed as Mean ±SD and percentages. Changes in the clinical and radiographic parameters from baseline to 3, 6 and 9 months post-surgery in both experimental and control groups were analyzed by Paired t- test for intra-group and inter-group comparisons were made by Unpaired t test. For all the tests a p- value of 0.05 or less was considered as statistically significant. These data were analyzed using statistical software (*SPSS v 10.5, IBM, Chicago)*.

Table I: Comparison Of Mean Values Of Plaque Index, Gingival Index And Gingival Bleeding Index At Different Intervals

	Experimental group					Control group				Difference between groups	
INDICES	Time Interval	Mean ±SD	Diff. From base-line	% Diff.	P-value	Mean ±SD	Diff. From base-line	% Diff	P-value	Diff in means	P-value

Plaque index	Baseline	0.70 ±0.09	-	-	-	0.69 ±0.09	-	-	-	-	-
	9months	0.27 ±0.07	0.43	61.4%	< 0.01	0.27 ±0.07	0.42	60.8%	<0.01	0.01	0.000 (HS)
Gingival index	Baseline	0.36 ±0.14	-	-	-	0.40 ±0.11	-	-	-	-	-
	9 months	0.14 ±0.05	0.22	61.1%	<0.01	0.15 ±0.05	0.25	25%	<0.01	0.03	0.000 (HS)
Gingival bleeding index	Baseline	49.16 ±9.0	-	-	-	52.5 ±14.2	-	-	-	-	
	9 months	13.3 ±4.9	35.8	72.9%	0.00	15.0 ±5.2	37.5	71.4%	<0.01	0.04	0.000 (HS)

Table – II: Comparison Of Mean Values Of Probing Pocket Depth Between Experimental And Control Groups At Different Intervals

Experimental group					**Control group**				**Difference between groups**	
Time interval	**Mean ±SD**	**Diff. From base-line**	**% Diff.**	**P-value**	**Mean ±SD**	**Diff. From base-line**	**% Diff**	**P-value**	**Diff in mean s**	**P-value**
Baseline	6.9 ±1.7	-	-	-	6.5 ±1.9	-	-	-	-	-
9months	4.8 ±3.01	2.1	30.4 %	<0.01	5.1 ±1.3	1.4	21.5 %	<0.01	0.7	<0.06 (NS)

Table III: Comparison Of Mean Values Of Clinical Attachment Level At Different Intervals

Experimental group					**Control group**				**Difference between groups**	
Time	**Mean**	**Diff.**	**%**	**P-**	**Mean**	**Diff.**	**%**	**P-**	**Diff**	**P-**

interval	±SD	From base-line	Diff.	value	±SD	From base-line	Diff	value	in means	value
Baseline	6.3 ±1.9			-	5.8 ±2.03			-		-
9months	5.3 ±2.1	1	15.8%	<0.01	4.8 ±2.4	1	17.2%	<0.01	0	1.0 (NS)

Table - IV: Comparison Of Mean Values Of Gingival Margin Position Between Experimental And Control Groups At Different Intervals

Experimental group					Control group				Difference between groups	
Time interval	Mean ±SD	Diff. From base-line	% Diff.	P-value	Mean ±SD	Diff. From base-line	% Diff	P-value	Diff in means	P-value
Baseline	0.91 ±1.31			-	0.91 ±0.90			-		-
9months	0.16 ±1.1	0.75	82.4%	<0.1	0.6 ±1.1	0.31	34%	<0.1	0.44	<0.05 (S)

Table V: Comparison Of Radiographic Changes Between Experimental And Control Groups At Different Intervals

Intra group							Inter group	
Experimental group				Control group				
Measurements	Mean ±SD	t-value	P-value	Mean ±SD	t-value	P-value	Mean difference	P-value
Initial defect depth(Q-R)	6.54 ±3.2		-	8.6 ±5.1	-	-	2.06	0.19(NS)
9months defect depth (Q^9-R^9)	3.8 ±2.2		-	7.4 ±5.4	-	-	3.60	0.21(NS)
Amount of defect fill	2.74 ±1.0	2.9	<0.5(S)	1.2 ±0.3	1.5	<0.1(S)	1.54	0.01(S)
Percentage fill of original defect	31.2 ±61.4	-	-	40.7 ±31.7			9.5	0.79(NS)
Change in alveolar crest	-0.58 ±3.57	0.46	0.66(NS)	1.35 ±4.74	0.81	0.45(NS)	1.93	0.34(NS)
Percentage	-6.99	-	-	0.33			1.03	<0.001(HS)

change in alveolar crest	±5.21			±0.64					
Percentage of original defect resolved	51.0 ±64.5	-	-	7.8 ±56.4			43.2	0.09(NS)	

DISCUSSION

Kataria et al 2016 conducted a study on autogenous bone graft for management of periodontal defects.The aim of this article is to present the use of autogenous bone graft for regenerative management of infrabony and furcation osseous defects. A trephine bur (Auto Chip Maker ®) was used to obtain autograft from the anterior mandibular region. It can be concluded that periodontal defects can be successfully treated with autogenous bone graft, which can be easily procured using newer trephine burs such as the Auto Chip Maker ® .[11]

Dan J et al (2012) conducted a study using amnion-chorion allograft barrier used for guided tissue regeneration treatment of periodontal intrabony defects. The results of this retrospetive observational report are promising and warrant additional controlled , long -term studies to further evaluate the effectiveness of ACM for combination GTR treatment of periodontal intrabony defects.[12]

Kiany F et al (2015) conducted a study on Amnion membrane as a novel barrier in the treatment of intrabony defects. The purpose of this 6-month randomized, controlled, blinded, clinical trial was to evaluate and compare the efficacy of amnion membrane (AM) with deproteinized bovine bone mineral (BBM) and a collagen membrane (CM) with BBM in guided tissue regeneration (GTR) for the treatment of intrabony periodontal defects and concluded that both AM and CM with BBM provided improvement of clinical periodontal parameters. AM did not induce significant gingival recession and is suggested as a new barrier membrane in GTR treatment.[13]

Due to lack of comparative studies on periodontal regeneration by Autogenous graft and Lyophilised Irradiated Amniotic membrane clinically and radiographically this study has been attempted.

RESULTS

All patients showed good clinical compliance and the healing period was uneventful for both the treated groups, without showing any signs of infection or complications. The results of the clinical parameters were recorded in Table I-V.

CONCLUSION

Regeneration of the periodontium has long been a challenge to the clinician. Various types of regenerative materials have been tested in the past decade. By virtue of the properties vested in Autogenous graft and Amniotic membrane, it may be possible to bridge the gap between ideal and effective treatment modalities.

The present study was done to evaluate the effectiveness of Autogenous graft and lyophilised irradiated Amniotic membrane in the treatment of intrabony defects. Autogenous graft in the treatment of intrabony defects has found to be effective, however with a combined effect of lyophilised irradiated Amniotic membrane have shown better results; wherein the results showed greater improvement in clinical and radiographical parameters in relation to the PD reduction, CAL gain and defect fill. Within the limitations of this study, it can be concluded that the use of Autogenous graft and lyophilised irradiated Amniotic membrane is a simplified, easy, fast and cost-effective.

However, long term and larger sample size studies need to be carried out to affirm the observations of this study.

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