

Treatment of Intrabony Defects by Drug Coated Membrane Vs Resorbable Membrane Only: A Clinical, Microbiological & Radiological Evaluation

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ABSTRACT

Aims: Barrier membranes used as Guided tissue regeneration (GTR) at operative site may be prone for microbial colonization. So, preoperative coating of these membranes with antibiotics like doxycycline may help to achieve better regeneration by hindering re-infection.

Materials & methods: 16 patients, 10 females (6 patients with bilateral defects & 4 patients with only one angular defect) & 6 males (2 patients with bilateral & 2 patients with only one angular defect) aged between 25 to 55 years with the mean age of 38 years with two or three walled infra bony defects were randomly selected, divided into two groups, group A & B. Patients in group A received doxycycline coated GTR (Healiguide) membrane which was incorporated over the intrabony defect after flap surgery, whereas group B patients received only GTR(Healiguide) membrane after the surgical debridement. Microbiologic assessment of Porphyromonas gingivalis (Pg) & Prevotella intermedia(Pi) levels were done at baseline, 6 and 12 weeks. Clinical parameters like Plaque index (PI), Probing depth (PD), Clinical attachment level (CAL), and radiographic evaluation with grid were done at baseline, 3 & 9 month intervals.

Results: There was a statistically significant reduction in, Pg levels ($p < 0.001$), Pi levels ($p < 0.001$), PI ($p = 0.004$), PD ($p < 0.001$), gain in CAL ($p = 0.02$) and defect fill ($p = 0.024$) in group A rather than group B.

Conclusion: Both the groups showed a significant improvement in microbiological, clinical & radiographic parameters, but group A showed statistically significant results.

Keywords: Infra bony defects, Antimicrobial agents, Membrane, Regeneration, Bone fill.

INTRODUCTION

Periodontal regeneration is defined as regeneration of the tooth's supporting tissues, including alveolar bone, periodontal ligament and cementum over previously diseased root surface¹.

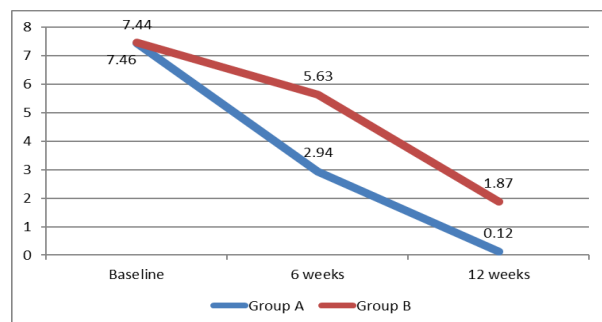
Guided tissue regeneration is a principle applied in periodontal therapy which aims at achieving predictable formation of new attachment apparatus. Melcher in 1976 who developed the theoretical principle related to guided tissue regeneration emphasized on the necessity to prevent the

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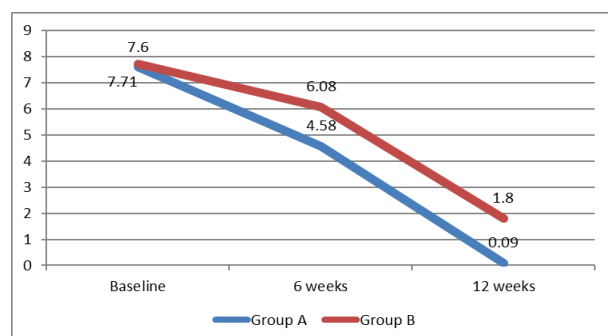
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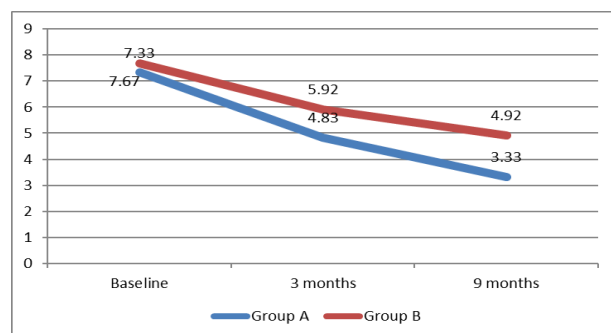
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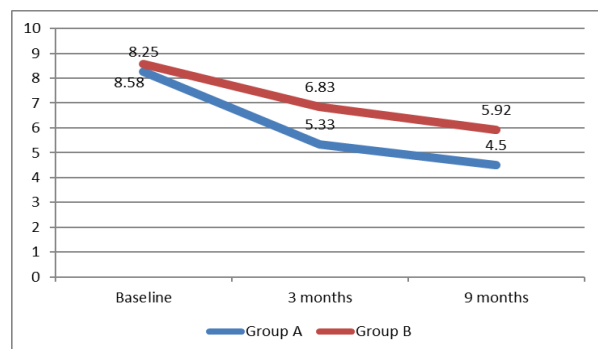
Graph 1: Comparison of changes in Pg levels at different intervals between two groups.



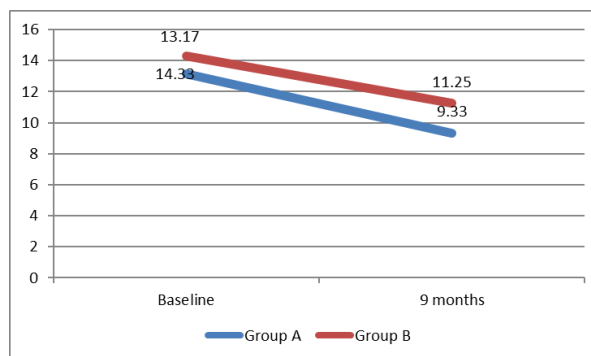
Graph 2: Comparison of changes in Pi levels at different intervals between two groups.



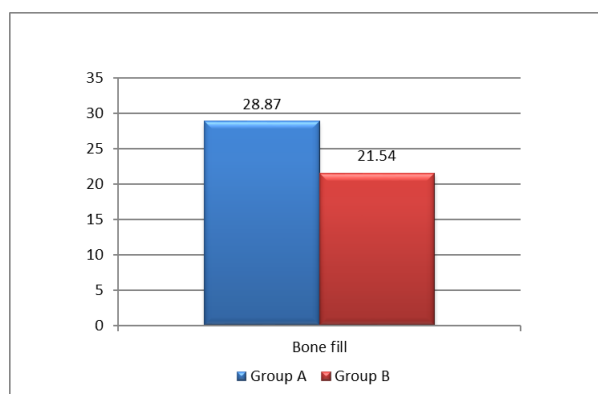
Graph 3: Comparison of changes in PD between two groups.



Graph 4: Comparison of changes in CAL between two Groups.



Graph 5: Comparison of changes in defect depth between two groups.



Graph 6: Comparison of changes in amount of bone fill between two groups.

migration of unwanted cell lines onto the surgical site to aid in the growth of desired tissues². This is achieved by maintaining the space for the surgical defect to heal as well as by initial stabilization of the clot³. Fibrin linkage with root surface is maintained by developing the new connective tissue formation, failure of this may results in the formation of long junctional epithelium. Numerous factors complicate the process of periodontal regeneration such as the presence of multiple specialized cell types and attachment complexes, stromal cellular interactions, diverse microbial flora and avascular tooth surfaces^{4,5}. Bacterial colonization of the membrane would result in improper healing and reduced regeneration⁶. Hence, coating the barrier membrane with a potential antimicrobial substance helps in achieving enhanced regeneration and complete asepis.

Doxycycline is potentially valuable antimicrobial agent and substantially available in gingival fluid which acts against numerous periodontal pathogens⁷. It demonstrates

Table 1: Pg & Pi levels at different intervals between two groups.

	Pg						Pi					
	Group A			Group B			Group A			Group B		
	BL	6W	12W	BL	6W	12W	BL	6W	12W	BL	6W	12W
Mean	7.44	2.94	0.12	7.46	5.63	1.87	7.60	4.58	0.09	7.71	6.08	1.80
Standard deviation	0.68	2.74	0.27	0.58	0.88	0.56	0.83	1.80	0.32	0.83	0.65	0.75
N	12			12			12			12		
p Value	<0.001			<0.001			<0.001			<0.001		
Post -hoc test	1>2>3			1>2>3			1>2>3			1>2>3		

Table 2: Clinical Parameters (PI, PD, CAL) values at different intervals between two groups.

	Group	PI			PD			CAL		
		0	3M	9	0	3M	9M	0	3M	9M
Mean	A	0.48	0.35	0.20	7.33	4.83	3.33	8.58	6.83	5.92
	B	0.53	0.41	0.33	7.67	5.92	4.92	8.25	5.33	4.50
Standard deviation	A	0.07	0.08	0.10	0.89	0.58	0.78	1.31	1.11	0.79
	B	0.09	0.08	0.12	0.98	1.00	0.90	1.14	0.78	1.24
N		12			12			12		
P Value	A	<0.001			<0.001			<0.001		
	B	<0.001			<0.001			<0.001		
Post hoc Test		1>2>3			1>2>3			1>2>3		

Table 3: Defect Depth between the two groups from baseline to 9 months.

Group		Mean	N	Std. Deviation	p-value
Group A	Defect depth-B	13.17	12	1.95	<0.001
	Defect depth-9m	9.33	12	1.72	
Group B	Defect depth-B	14.33	12	2.61	<0.001
	Defect depth-9m	11.25	12	2.18	

anticollagenolytic & antiproteolytic properties there by strengthening host resistance by inhibiting the production of collagenase from polymorphonuclear leukocytes that aid in osseous regeneration⁸. Additionally, it is substantive to both cementum and dentin suggesting that root surfaces could serve as substrates for deposition and subsequent slow release⁹.

Healiguide, a type I bovine collagen membrane, and a bio-resorbable material used in this study, facilitates fibrogenesis over

osteoconduction and enhances regeneration of periodontal defects. This membrane is superior to other collagen membranes because it is isomer pristine and has better bio-compatibility¹⁰.

MATERIALS AND METHODS

This prospective, clinical interventional study was carried out in the Department of Periodontics, in a tertiary referral care hospital in Hyderabad. The study samples were randomly divided by investigator KRR. Clinical parameters

like PI (Sillness & loe), PD, CAL & microbiological assessment of Pg & Pi levels (pooled plaque samples) as well as radiological defect depth (IOPA with grid) were recorded by investigator HB. All the patients underwent scaling & root planning at baseline by investigator HB who was blinded to the randomization process and who also performed the surgical interventions. Ethical clearance was obtained from the institutional review board and written informed consent was taken from all the patients.

Inclusion criteria:

1. Patients with chronic periodontitis with presence of two or three walled intrabony defects.
2. Probing pocket depth ≥ 5 mm with radiographic evidence of angular bone loss.

Exclusion criteria:

1. Patients who have undergone periodontal therapy 3 months prior to the study.
2. Systemically compromised patients on medications
3. Smokers
4. Pregnant women and lactating mothers.
5. History of antibiotics one month prior to the study
6. Patients who did not comply with oral hygiene maintenance.
7. Teeth with mobility.

Clinical periodontal parameters were measured with Williams graduated periodontal probe, along with the occlusal stent. Microbiological assessment: Sub gingival plaque samples were collected from the test and control sites and processed in the following manner: 3 paper points (No 30, Johnson Fine Absorbent points. Johnson & Johnson, East Windsor, NJ) were inserted into the pocket, kept in place for 1 minute and removed. The samples were then transferred to a transport medium containing, Robertson's cooked meat broth (RCM broth) (Fig 1) and were sealed immediately to create strict anaerobic conditions and transported to the laboratory. Within 8 hrs broth was transferred to anaerobic jars which contained RCM agar and incubated at 37°C for 24 hours where turbid appearance indicated the presence or growth of culture. (Fig 2) Further, Subculture was made on

blood agar (5% sheep blood) and brain, heart infusion agar supplemented with vitamin K (0.1mg/L) and haemin (1g/ml). The plates were incubated at 37°C for 72 hours under strict anaerobic conditions and transferred to anaerobic jars supplemented with 10% CO₂ where the anaerobic conditions were monitored with the help of biological indicator (*Pseudomonas aeruginosa* and chemical indicator methylene blue). After incubation these anaerobic cultures were identified through gram staining, where brown color and black colored colonies (Fig 3) indicated the presence of Pi & Pg respectively.

Radiological assessment: Bone fill was measured with intra oral radiographs along with grid, in mm markings. Bone fill was evaluated from the adjacent cuspal tip to the most apical part of the base of the defect (Fig 4, 4a)

Preparation of the membrane coating:

Healiguide,TM a resorbable collagen membrane was shaped to meet requirements of the recipient site. Coating of membrane was performed by dissolving 100mg of doxycycline hydrochloride dispersible tablet in 0.4ml of saline. This paste was smeared onto the tooth-facing surface of the collagen membrane prior to its placement in the infrabony defect.

Surgical procedure

Full thickness flap was raised and de granulated to expose the defect (Fig 5, 5a). Group A patients received prefabricated Doxycycline coated membrane which was placed according to defect morphology with the help of membrane holder (Fig 6). The membrane was stabilized to the tooth using sling sutures (Resorbable 3.0 vicryl). Group B samples received only membrane without drug coating (Fig 6a) and sutures were given (Fig 7, 7a). Anti inflammatory drugs (etorocoxib 90mg) were prescribed for three days.

Statistical analysis

One way analysis of variance (ANOVA) followed by post-hoc bonferroni test was used to evaluate significant difference in intra groups by using SPSS version 17. Paired t test was used to assess the inter group values. A Probability (p)

value of <0.05 was considered to be statistically significant.

RESULTS

On analyzing the microbiologic counts, the mean reduction of Pg from baseline to 12 weeks in group A was 7.44 ± 0.68 , 2.94 ± 2.74 & 0.12 ± 0.27 and in group B, 7.46 ± 0.58 , 5.63 ± 0.88 & 1.87 ± 0.56 respectively, with the p value 0.001 which was considered statistically significant. Similarly, the mean reduction of Pi from baseline to 12 weeks in group A was 7.60 ± 0.83 , 4.58 ± 1.80 & 0.09 ± 0.32 and 7.71 ± 0.83 , 6.08 ± 0.65 & 1.80 ± 0.75 in group B respectively with p value 0.001 which was considered significant (Table I, Graph I&II).

The mean reduction of PI from baseline to 9 months in group A was 0.48 ± 0.07 , 0.35 ± 0.08 & 0.20 ± 0.10 and in group B, 0.53 ± 0.09 , 0.41 ± 0.08 & 0.33 ± 0.12 respectively with p value 0.001 which was statistically significant (Table II).

The mean reduction of PD in group A was 7.33 ± 0.89 , 4.83 ± 0.58 & 3.33 ± 0.90 mm and in group B, 7.67 ± 0.98 , 5.92 ± 1.00 , 4.92 ± 0.90 mm respectively, with p value 0.001 which was statistically significant (Table II, Graph III). With respect to CAL, group A showed 8.58 ± 1.31 , 6.83 ± 1.11 & 5.92 ± 1.24 mm and in group B 8.25 ± 1.14 , 5.33 ± 0.78 & 4.50 ± 1.24 mm gain with p value 0.001. Statistically significant attachment gain was seen (Table II, Graph IV). Regarding the linear bone fill, the mean reduction in the depth of defect from baseline to 9 months in group A was 13.17 ± 1.95 & 9.33 ± 1.72 mm and in group B was 14.33 ± 2.61 & 11.25 ± 2.18 mm respectively with p value 0.001 which was statistically significant. But bone fill was better in group A. (Table III, Graph V) (Fig. 8,8a)

DISCUSSION

Periodontal regeneration is a favourable outcome which every clinician would want to achieve. There are many number of treatment modalities for regeneration out of which the Guided tissue regeneration principle, stands apart as it has been applied for a long time with successful results. However, it was observed that microbial colonization of barrier materials used in GTR procedures during the periodontal surgery might hamper the post surgical healing which can be

prevented by priming the membrane with antibiotics like doxycycline. Studies have shown that among all the antibiotics, doxycycline has superior properties like antimicrobial as well as anti-collagenolytic, anti-inflammatory, osteoclast-inhibitory, fibroblast-stimulatory and intrinsic host-modulatory action¹¹. Doxycycline might initiate demineralization, which would probably result in the release of some osteogenic factors, such as Transforming Growth Factor (TGF- β), Insulin like Growth Factor (IGF) and Bone Morphogenic Proteins (BMPs) into the surrounding tissues to trigger the bone induction effects¹². Hence, in this study doxycycline was used to coat the GTR membrane.

In this study amongst the parameters assessed clinically the PI scores,¹³ Pocket probing depth and CAL gain from baseline to 9 months, showed statistically significant results in both the groups but more significant in group A with p value 0.001. These results are in accordance with studies done by Mombelli et al¹⁴ Zarkesh et al¹⁵ Chang et al¹² related to pocket depth reduction and Cortellini P et al^{16,18}, Eickholz P¹⁷ related to CAL Gain.

Microbial analysis was done consecutively at base line, 6 weeks and twelve weeks intervals only because, when doxycycline is used as a local drug delivery system its action lasts at the tissue site from days to weeks only. This is proven by many in vitro and in vivo studies.

In a study done by Demirel¹⁸ et al on doxycycline coated specimens it was observed that the antibacterial effect of tissues treated with concentration of 100mg/ml persisted for 14 days to several days, implying that biologically active form of doxycycline is available in tissues for very short time. And also, at that high concentration, inhibition zones of few microorganisms were seen at all time intervals, among them Pg has been observed to be more sensitive to doxycycline¹⁸. In this study statistically significant reduction of Pi as well as Pg levels were seen in group A that is doxycycline coated membrane with p value 0.001.

The defect fill in both the groups were statistically significant with p value 0.001, but more defect fill was observed in group A. Similar results were found with studies done by Chung CP et al²⁰, Chao-Yi Chang et al¹². The overall inference drawn

from this study is almost similar significant results were found with all parameters in both doxycycline incorporated and not incorporated groups. This study was supported by the study done by Chaturvedi et al¹⁹.

Shortcomings of the study:

In this study conventional radiographs were used, though conventional radiography is simple, easy and reasonable, sensitivity and geometric distortions are the limitations in this study, Small sample size is also another drawback of this study.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

CONCLUSION

There are certain drugs such as tetracycline which have been found in higher concentrations in the crevicular fluid similar to serum after same oral dose. The drug can bind to tooth surface from which it is released in active form. Therefore use of such types of drugs are beneficial in treatment of periodontal diseases. Substantivity of doxycycline hydrochloride as a substrate, when deposited on periodontally diseased root surfaces may last long if used in the form of local drug. In this study, doxycycline as a drug coated membrane has shown satisfactory results in regeneration of periodontal defects, however many more longitudinal studies using doxycycline with a larger sample size have to be done to prove the beneficial effects of the drug.

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