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ORIGINAL ARTICLE

Combination of bone allograft, barrier membrane and doxycycline in the treatment of infrabony periodontal defects: A comparative trial

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Abstract *Aim:* The purpose of the present study was to compare the regenerative potential of noncontained periodontal infrabony defects treated with decalcified freeze-dried bone allograft (DFDBA) and barrier membrane with or without local doxycycline.

Methods: This study included 48 one- or two-wall infrabony defects from 24 patients (age: 30–65 years) seeking treatment for chronic periodontitis. Defects were randomly divided into two groups and were treated with a combination of DFDBA and barrier membrane, either alone (combined treatment group) or with local doxycycline (combined treatment + doxycycline group). At baseline (before surgery) and 3 and 6 months after surgery, the pocket probing depth (PPD), clinical attachment level (CAL), radiological bone fill (RBF), and alveolar height reduction (AHR) were recorded. Analysis of variance and the Newman–Keuls post hoc test were used for statistical analysis. A two-tailed p-value of less than 0.05 was considered to be statistically significant.

Results: In the combined treatment group, the PPD reduction was 2.00 ± 0.38 mm (32%), CAL gain was 1.25 ± 0.31 mm (17.9%), and RBF was 0.75 ± 0.31 mm (20.7%) after 6 months. In the combined treatment + doxycycline group, these values were 2.75 ± 0.37 mm (44%), 1.5 ± 0.27 mm (21.1%), and 1.13 ± 0.23 mm (28.1%), respectively. AHR values for the groups without and with doxycycline were 12.5% and 9.4%, respectively.

Conclusion: There was no significant difference in the regeneration of noncontained periodontal infrabony defects between groups treated with DFDBA and barrier membrane with or without doxycycline.

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1. Introduction

Bone grafts can be used for reconstructing periodontal osseous defects and achieving new attachment for connective tissue fibers. Among the available graft materials, only autogenous bone and decalcified freeze-dried bone allograft (DFDBA)

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have histological evidence supporting their use in humans as regenerative materials (Darby, 2011; Rosen et al., 2000). In addition to patient-specific characteristics (plaque control, smoking habits, wound healing potential), factors related to the defect morphology, particularly the morphology of the remaining wall in the defect, may play important roles in regenerative outcomes (Cortellini et al., 1998; Park et al., 2014).

DFDBA has osteoinductive activity because it contains bone morphogenic proteins (BMPs). Decalcification of the graft exposes and may activate bone-inductive proteins in the bone matrix (Behfarnia et al., 2012). Studies have confirmed that the use of DFDBA can lead to the formation of new attachments (Darby, 2011). There are several advantages to using bone grafts with guided tissue regeneration (GTR). For example, bone grafts prevent membrane collapse inside the defect, improve space maintenance and clot stabilization, and facilitate the proliferation of osteogenic progenitor cells (Paolantonio et al., 2010; Sculean et al., 2008). Polylactic acid/polyglycolic acid (PLA/PGA) membrane is a synthetic bioabsorbable barrier membrane made from a copolymer of glycolide and lactide. Many studies have used PLA/PGA membrane in the treatment of infrabony defects (Aimetti et al., 2005; Kim et al., 2002).

Doxycycline facilitates regenerative therapy by initiating demineralization of the bone surface layer. This demineralization results in the release of osteogenic factors, such as transforming growth factor (TGF), insulin-like growth factor, and BMPs, which trigger bone induction (Kaur and Sikri, 2013). Doxycycline has anticollagenolytic and antiproteolytic properties that enhance the bone-forming ability via osteoblast cell chemotaxis and reduced bone resorption (Chaturvedi et al., 2008).

It could be beneficial to use local doxycycline with a bone graft in anatomically unfavorable infrabony defects, which are more vulnerable to oral contamination due to their noncontained nature and have inherently less osteogenic potential due to the smaller amount of remaining wall. However, little data are available comparing the combined use of DFDBA plus barrier membrane with and without local doxycycline in human periodontal infrabony defects. Therefore, the present study was undertaken to explore the beneficial effects of combined therapy with local doxycycline in noncontained periodontal infrabony osseous defects.

2. Materials and methods

2.1. Patients

This study included 48 infrabony periodontal defects (27 two-wall and 21 one-wall defects) in 24 patients (14 males and 10 females; age: 30–65 years) who were seen at the Outpatient Department of Periodontics, Aligarh Muslim University, Aligarh for treatment of moderate to severe chronic periodontitis. Inclusion criteria were the presence of contralateral one- and two-wall intraosseous infrabony defects with a pocket probing depth (PPD) of 5 mm or more and a defect depth (as assessed through bone probing) of 3 mm or more, as well as fewer than 20% of gingival sites exhibiting bacterial plaque (%PL+) or bleeding on probing (%BOP+). Exclusion criteria were any systemic disease, medication use, pregnancy or lactation, smoking habit, previous periodontal treatment, and furcation (according to Glickman). After recruitment of patients, the study protocol, risks, benefits, and procedures were explained, and written informed consent was obtained from every patient. All examinations, treatments, and procedures associated with this study followed the principles of the Declaration of Helsinki. The study was reviewed and approved by the ethics committee of Aligarh Muslim University.

2.2. Study design

The study was designed as a randomized, double-blinded study comparing the periodontal outcomes obtained when using DFDBA plus a barrier membrane with or without local doxycycline in the treatment of intrabony defects. A split-mouth design was used. Defects were randomly divided into two groups by a computer-generated system, according to treatment. The control group was treated with DFDBA and a barrier membrane (combined treatment group). The test group was treated with DFDBA, barrier membrane, and local doxycycline (combined treatment + doxycycline group). Defects were analyzed clinically and radiologically at baseline (before surgery) and 3 and 6 months after regenerative surgery by a single investigator for each surgical site.

2.3. Clinical and radiographic parameters

Every patient received a complete periodontal examination, oral hygiene instructions, and a thorough scaling and root planing session prior to surgery. Nonsurgical therapy and presurgery recordings were performed 4 weeks before surgery. Presurgical evaluation included %PL+ or %BOP+ sites, PPD, and clinical attachment level (CAL). Parameters associated with the defects were not significantly different between the control and test groups. PPD and CAL were recorded to the nearest millimeter with the help of a manual UNC-15 probe (Hu-Friedy Mfg. Inc., Chicago, IL) at the deepest point of the periodontal pocket, from the vertical groove in the acrylic stent on the occlusal surface.

Radiographic parameters included the radiographic bone fill (RBF) and resorption of alveolar bone height (alveolar height reduction, AHR). The distance from the reference point (radiographic cemento-enamel junction, CEJ) to the base of the defect (BD) and to the crest of the bone (CB), and the distance from the CB to the BD (radiologic defect depth, RDD) were measured. RBF was defined as the difference between the pre- and post-treatment measurements of RDD. AHR was defined as the difference between the pre- and post-treatment measurements of the distance from the radiologic CEJ to the alveolar bone height (ABH). Intraoral periapical radiographs were taken with the parallel cone technique and a customized film holder. An effort was made to obtain similar projection geometries and optical densities for the pre- and post-operative radiographs. Parameters were recorded to the nearest millimeter with a radiological grid (1×1 mm).

2.4. Surgical protocol

After administration of local anesthesia, intrasulcular incisions were made, and full-thickness mucoperiosteum flaps were

Figure 1 Pre operative pocket probing depth in DFDBA + barrier membrane group.

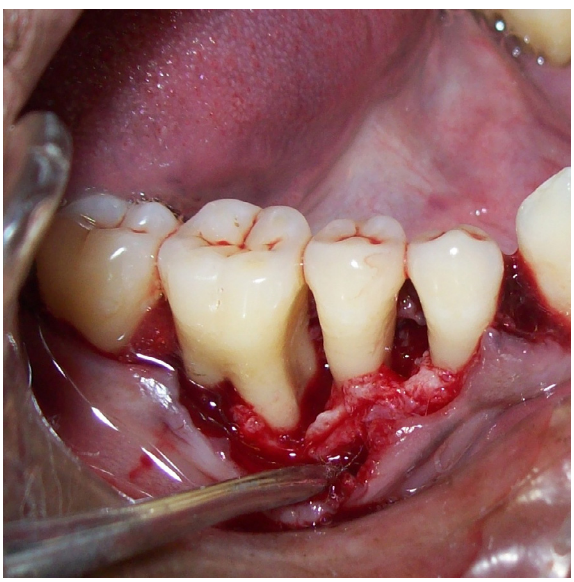

Figure 2 Infra bony defect mesial to 2nd premolar.

reflected buccally and lingually. Care was taken to preserve the interdental papilla. Thorough debridement was carried out in the infrabony defect areas.

For the combined treatment + doxycycline group, DFDBA (LifeNet Health, Virginia Beach, VA) and doxycycline pellets were mixed at a 1:4 volume ratio (Evanas et al., 1989) of doxycycline to DFDBA. The mixture was reconstituted with sterile saline water and placed in the defect up to the alveolar crest. A fresh PLA/PGA membrane (Polyglyctin 910, Ethicon, Johnson & Johnson, Norderstedt, Germany) was trimmed according to the defect morphology and adapted over the defect, which was filled with graft beyond 2–3 mm of the perilesional bone margin both apically and laterally. For the combined treatment group, the same procedure was

Figure 3 Barrier membrane placement over bone graft in defect area.

Figure 4 Pocket probing depth after 6 months in DFDBA + barrier membrane group.

performed by mixing bone graft with placebo at the same ratio as doxycycline.

For flap closure, 3–0 black braided silk sutures were used. Periodontal dressing was utilized for wound stabilization and patient comfort (Figs. 1–6). The postoperative regimen included verbal and written instructions. Patients were prescribed 500 mg of amoxicillin and 125 mg of clavulanic acid three times a day for 5 days, and 0.12% chlorhexidine gluconate mouth rinse twice daily for 2 weeks. Sutures were removed after 7 days. Patients were placed on a maintenance program of biweekly professional tooth cleaning.

2.5. Statistical analysis

Groups were compared by repeated-measures analysis of variance (ANOVA). The significance of mean differences within and between groups was determined by the Newman–Keuls

Figure 5 Pre operative radiograph of DFDBA + barrier membrane group.

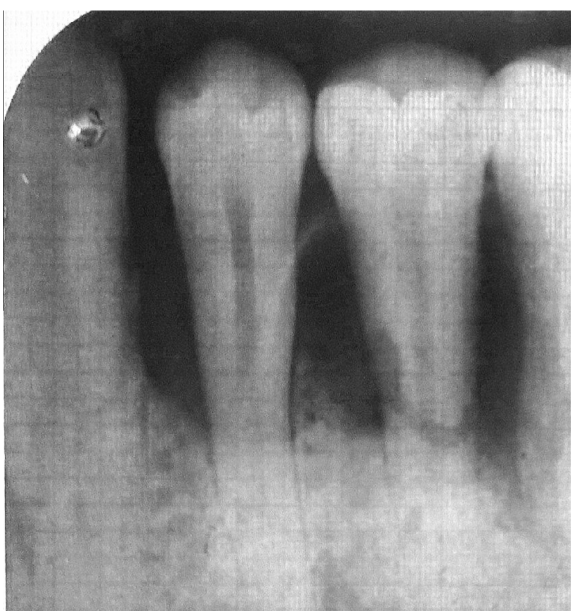

Figure 6 Post operative radiograph of DFDBA + barrier membrane group.

post hoc test. A two-tailed ($\alpha = 2$) p-value of less than 0.05 was considered to be statistically significant.

3. Results

All patients maintained a high standard of plaque control throughout the study. All defects healed uneventfully, with no clinical or radiographic indication of ankylosis.

In the combined treatment group, the %PL + and %BOP + sites were 13.8% and 14.9%, respectively, at baseline, and 13.3% and 14.4%, respectively, after 6 months ($p > 0.05$). For the combined treatment + doxycycline group, the %PL + and %BOP + sites were 13.9% and 14.5%, respectively, at baseline, and 12.5% and 13.3%, respectively, after 6 months ($p > 0.05$). Comparing the clinical and radiographic measurements at different time points, statistically significant improvements of soft tissue parameters were obtained between baseline and 3 or 6 months. Significant changes in hard tissue parameters were found between 3 and 6 months.

For the control group, the PPD reduction was 2.00 ± 0.38 mm (32%), CAL gain was 1.25 ± 0.31 mm (17.9%), and RBF was 0.75 ± 0.31 mm (20.7%). For the test group, these values were 2.75 ± 0.37 mm (44%), 1.5 ± 0.27 mm (21.1%), and 1.13 ± 0.23 mm (28.1%), respectively for the comparison of base line and 6 months. The AHR values in the groups without and with doxycycline were 0.38 and 0.37 mm, respectively. These results and their significance (p-values) at different time intervals are summarized in Table 1. There were no significant differences in PPD, CAL, RDD, or ABH between the control and test groups at any time point (Table 2).

For two-wall defects, changes of 31.4% and 46.1% in PPD, 17.5% and 21.8% in CAL, 27.7% and 27.7% in RDD, and 10% and 16.6% in ABH were recorded for the control and test groups, respectively. For one-wall defects, changes of 35.4% and 41.7% in PPD, 18.8% and 20.0% in CAL, 9.0% and 28.5% in RDD, and 18.3% and 5.88% in ABH were recorded for the control and test groups, respectively (Table 3).

4. Discussion

Regenerative outcomes of noncontained periodontal infrabony defects were not significantly different between groups treated with DFDBA and barrier membrane with or without local doxycycline. Both groups showed significant improvements in parameters after 6 months compared to baseline. Better results for one- and two-wall defects were obtained in the group treated with doxycycline. Two-wall defects showed

Table 1 PPD, CAL, RDD, ABH (mm) summary (Mean ± SE, $n = 24$), and significance (p value) of mean difference between groups at three different periods.

DFDBA + barrier membrane group				
	Baseline	3 month	6 month	Mean change (%)
PPD	6.25 ± 0.67	4.25 ± 0.59	4.25 ± 0.56	32.0
CAL	7.00 ± 0.71	5.88 ± 0.58	5.75 ± 0.59	17.9
RDD	3.63 ± 0.50	3.38 ± 0.42	2.88 ± 0.35	20.7
ABH	2.63 ± 0.50	2.88 ± 0.52	3.00 ± 0.60	12.5
DFDBA + barrier membrane + doxycycline group				
PPD	6.25 ± 0.62	3.38 ± 0.18	3.50 ± 0.27	44.0
CAL	7.13 ± 0.81	5.63 ± 0.56	5.63 ± 0.56	21.1
RDD	4.00 ± 0.42	3.75 ± 0.53	2.88 ± 0.40	28.1
ABH	3.63 ± 0.94	3.75 ± 0.88	4.00 ± 0.98	9.4
Significance (p value) of mean difference between without vs. with doxycycline group				
PPD	1.000	0.748	0.732	–
CAL	0.884	0.991	0.884	–
RDD	0.793	0.914	1.000	–
ABH	0.497	0.497	0.178	–

PPD – pocket probing depth; CAL – clinical attachment level; RDD – radiographic defect depth; ABH – alveolar bone height; SE – standard error.

Table 2 For each group, significance (p value) of mean difference in PPD, CAL, RDD, ABH between the periods (within groups).

	PPD	CAL	RDD	ABH
DFDBA+ barrier membrane group				
Baseline vs. 3 months	0.001	0.001	0.560	0.524
Baseline vs. 6 months	0.001	0.001	0.048	0.258
3 month vs. 6 months	1.000	0.605	0.254	0.497
DFDBA+ barrier membrane group doxycycline				
Baseline vs. 3 months	0.001	0.001	0.307	0.497
Baseline vs. 6 months	0.001	0.001	0.001	0.111
3 month vs. 6 months	0.937	1.000	0.010	0.178

PPD – pocket probing depth; CAL – clinical attachment level; RDD – radiographic defect depth; ABH – alveolar bone height.

Table 3 Distribution and data for 2 wall and 1 wall defects.

	DFDBA+ barrier membrane group			DFDBA+ barrier membrane + doxycycline group		
	Two wall defects (14)			Two wall defects (13)		
(mm)	Base line	6 months	Change (%)	Base line	6 months	Change (%)
PPD	7.00	4.80	31.4	6.50	3.50	46.1
CAL	8.00	6.60	17.5	8.00	6.25	21.8
RDD	3.60	2.60	27.7	4.50	3.25	27.7
ABH	2.00	2.20	10	3.00	2.50	16.6
	One wall defects (10)			*One wall defects (11)*		
PPD	5.00	3.33	35.4	6.00	3.50	41.7
CAL	5.33	4.33	18.8	6.25	5.00	20.0
RDD	3.66	3.33	9.0	3.50	2.50	28.5
ABH	3.66	4.33	18.3	4.25	4.50	5.88

PPD – pocket probing depth; CAL – clinical attachment level; RDD – radiographic defect depth; ABH – alveolar bone height.

greater bone fill and less crestal resorption with both treatment modalities.

Our findings are in agreement with previous studies reporting significant improvements in the clinical parameters of infrabony defects treated by DFDBA with tetracycline (Drury and Yukna, 1991; Mabry et al., 1985; Masters et al., 1996; Waleed et al., 1989). The biological rationale for using doxycycline as an adjunctive to regenerative therapy is its antimicrobial, anticollagenolytic, and fibroblast-stimulating activities. The antimicrobial component makes doxycycline the drug of choice in periodontal regenerative therapy. The anticollagenolytic effect may act to inhibit collagenase and other host-derived matrix metalloproteinases that are responsible for alveolar bone resorption (Chaturvedi et al., 2008; Kaur and Sikri, 2013). Doxycycline protects alpha-1 proteinase inhibitor from proteolytic inactivation in the gingival crevicular fluid, and inhibits the production and scavenging of reactive oxygen radicals generated by neutrophils. Increased fibronectin binding to tetracycline-conditioned roots promotes the attachment of fibroblasts and inhibits epithelial cell attachment (Lee et al., 1997; Takahashi et al., 2006).

Studies have repeatedly demonstrated that using bone grafts combined with barrier membranes improves the regenerative parameters of noncontained infrabony periodontal defects (Chen et al., 1995; Lundgren and Slotte, 1999; Paolantonio, 2002; Trejo et al., 2000). In contrast to our results, Mellado et al. (1995) found a nonsignificant difference in new bone formation. The osteoinductive property of DFDBA is due to BMPs, which are members of the TGF-β super family. BMPs are powerful inducers of endochondral bone differentiation and act as soluble signals of tissue morphogenesis, sculpting the multicellular mineralized structures of periodontal tissues by inserting functionally oriented periodontal ligament fibers into the newly formed cementum (Chen et al., 2004; Yukna and Vastardis, 2005). PLA/PGA membrane is a synthetic bioabsorbable barrier membrane made from a copolymer of glycolide and lactide. This membrane is broken down by hydrolytic degradation. The use of bioresorbable membranes prevents the need of a second surgery for membrane removal. Many studies have used PLA/PGA membrane in the treatment of infrabony defects (Aimetti et al., 2005; Kim et al., 2002).

Adequate results have been reported on the association between the number of remaining defect-containing bony walls and the regenerative potential of periodontal infrabony defects. We selected one- and two-wall periodontal infrabony defects because three-wall infrabony defects have been reported to heal more favorably due to the adequate source of osteoprogenitor cells (Kornman and Robertson, 2000; Tonetti et al., 1993). Unfavorable defects, when present, are more exchangeable and susceptible to the oral environment. Thus, filling materials would have a greater chance to becoming contaminated, leading to an incomplete bone fill (Aimetti et al., 2005; Kornman and Robertson, 2000; Tonetti et al., 1993). Local doxycycline was added to the bone graft to overcome these shortcomings. The one- and two-wall defects showed less support to the GTR membrane; therefore, we used filling material beneath the membrane for better positioning of the barrier membrane.

The results of our small cohort study are less striking than those of previous studies, perhaps due to variations in several factors (e.g., patient selection, defect characteristics, data collection procedures, biochemical characteristics of grafted materials, surgery, and patient attitudes toward treatment) that influence the extent of clinical attachment gain and bone growth after grafting (Kornman and Robertson, 2000; Tonetti et al., 1993). All surgeries were performed by a single well-experienced periodontist to minimize interoperator variability. Furthermore, the regenerative potential of DFDBA therapy depends on the osteoinductivity of the bone graft samples, which may vary based on donor age, previous pathology and/or drug therapy, genetic variation, and the length of time required to harvest the cadaver bone (Jergesen et al., 1991; Schwartz et al., 1996). Finally, the validity of this study would be improved if the defect width had been considered. However, it would have been very difficult to obtain similar defects with standardized depths, widths, and morphologies.

5. Conclusion

For periodontal regeneration of noncontained infrabony defects, there were no significant clinical or radiologic differences between defects treated with DFDBA and barrier membrane with or without local doxycycline.

Conflict of interest

The authors have no conflict of interest to declare.

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