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Guided Tissue Regeneration using an Equine Bio-absorbable Collagen Membrane with or without Equine Bone Graft in the Treatment of Intrabony Defects in Patients with Aggressive Periodontitis Results of 18 month.

***Mahmoud Helmy Belal**

Damanhour University, Egypt

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***Corresponding author:** Mahmoud Helmy Belal, Department of Oral Medicine, Periodontology, Oral Diagnosis & Radiology, Faculty of Dentistry, Damanhour University, Damanhour, Al-Gomhouria Street, Egypt, Tel: 2-010-6571-656; Email: mahmoud_672000@yahoo.com

Abstract

Background: Successful periodontal regeneration is considered a gold standard for periodontists. Several GTR materials and bone grafts have been attempted but showed variable success rates.

Objective: The present randomized clinical and radiographic study was undertaken to compare the effectiveness of guided tissue regeneration (GTR) by using equine bioabsorbable collagen membrane with or without equine bone graft in the treatment of periodontal intrabony defects affected by aggressive periodontitis.

Materials and Methods: Ten systemically healthy patients with 20 periodontal intrabony defects were enrolled as a split mouth design. These defects were affected with aggressive periodontitis. The recorded measurements included plaque index, gingival bleeding index, gingival recession, PPD, clinical attachment level, radiographic defect depth and radiographic density. The defects were randomly distributed either as a control group (equine bioabsorbable collagen membrane alone) or a test group (equine bioabsorbable collagen membrane combined with equine bone graft).

Results: The inter- & intra-group comparison showed no significant differences between studied groups nor within each group for these clinical parameters (PI, BI & GR), except only gingival recession within group 1. On contrary, the inter- & intra-group comparison showed significant differences between studied groups and within each one for these parameters (PPD, CAL, RDD & RD).

At 18-month examination, PPD reduction was significantly greater in GTR + bone graft group (3.80±1.33 mm) compared with GTR group (2.60±1.57 mm), and clinical attachment level gain were 3.60±1.15 and 2.20±1.26 respectively. Radiographic DD reduction was similarly greater in GTR + bone graft group (3.30 ± 0.84 mm) compared with GTR group (2.40±1.09 mm). Also, the change in the radiographic density indicated a significant greater gain of mean gray level as (19.90 ± 16.00) in group 2 whereas 7.10±10.65 in group 1.

Conclusion: Use of GTR bioresorbable membrane with bone graft showed significant improved outcomes when compared to use of bioresorbable membrane in treating aggressive periodontitis. However, the studied groups showed significant improvement within each group when baseline & 18 month data were compared.

Keywords: Aggressive Periodontitis; Bone Graft; Guided Tissue Regeneration; Intrabony Defect

Abbreviations: AP: Aggressive Periodontitis; GTR: Guided Tissue Regeneration; CAL: Clinical Attachment Level; PPD: Probing Pocket Depth; GR: Gingival Recession; BI: Bleeding Index; PI: Plaque Index; RD: Radiographic Density; RDD: Radiographic Defect Depth; CBR: Crestal Bone Resorption; AC: Alveolar Crest; CEJ: Cementoenamel Junction; BD: Base of the Defect

Introduction

The aim of successful periodontal therapy is a trial to arrest inflammatory process, suppress microbial growth, control infection and attempt to restore the tissues destroyed due to periodontal disease. Different regenerative techniques may be used for such purposes, but the treatment or elimination of the infection should be the most important goal especially in patients having aggressive periodontitis [1,2].

Aggressive periodontitis (AP) is characterized by severe periodontal destruction within a relatively short period of time. It

has some etiologic agents capable of causing clinically detectable diseased levels that may negatively hazard oral healthy condition and alter daily life style of such patients. AP has less occurrence among periodontitis patients and few studies have evaluated some treatment protocols [3].

The predictable complete periodontal regeneration remains a major goal in the planned therapy. Despite several procedures such as usage of guided tissue regeneration (GTR), grafting materials, growth factors and/or host modulating agents have been attempted, the outcomes are not always predictable [4-6]. However, there is a great variation caused by many factors, for example; type of periodontitis, patient characteristics, anatomy of defect site and the surgical intervention [7]. The treatment of aggressive type of periodontitis has a great challenge to many clinicians.

In general, the most successful documentation of periodontal regeneration is GTR since it acts as an effective principal therapy for the treatment of different anatomic defects associated with periodontitis [5]. Some earlier animal [8,9] and human studies [10,11] indicated a predictable reconstruction of the periodontium by using either non-bioabsorbable or bioabsorbable membranes [12]. However, bioabsorbable GTR membranes were developed to avoid the second surgery needed to retrieve the non-resorbable barrier [13-16]. These bioabsorbable devices have two main products, natural (collagen membrane) and synthetic (copolymers) like Guidor, Vicryl periodontal mesh, Resolut and Atrisorb GTR barriers.

Use of GTR through the use of equine barrier and equine bone graft material showed a favorable clinical outcome and an effective periodontal therapy in the regenerative treatment of intrabony defects [17]. Also, these equine collagen membranes and equine bone acts as an effective therapy for guided bone regeneration in the treatment of bone defect consequent to removal of periapical cyst in clinical & histological report [18].

Some previous literature reports [13-16] were found, in which the efficacy of bioabsorbable membranes alone or combined with graft materials were evaluated and compared for regenerative purposes. However, to our knowledge, there are no available studies comparing the efficacy of using an equine bioabsorbable collagen barrier (Biocollagen®) alone or combined with equine graft (Bio-Gen®), in treating intrabony defects of aggressive periodontitis.

Subjects and Methods

Inclusion criteria

a) Aggressive periodontitis.

b) Intrabony periodontal defect sites with probing pocket depth > 5mm, as assessed by clinical and radiographic evaluation.

c) Radiographic evidence of vertical/angular bone loss.

d) Age ranged from 17 to 37 years.

e) Good general health.

Exclusion criteria

a. Periodontal treatment received during the last 6 months at least.

b. Hopeless teeth or evident grade-III mobile teeth.

c. Any relevant systemic diseases.

d. Smokers and/or alcoholics.

e. Pregnancy and/or lactation for female patients.

f. Hypersensitivity to any of the tested research materials.

Study Design

Ten patients (seven males & three females) were selected to be enrolled in this study and gathered from the out-patient clinic of the Department of Oral Medicine, Periodontology, Oral Diagnosis & Radiology. Verbal and written informed consent forms were obtained from all subjects and an ethical clearance was also get from the institution.

Ten patients with bilateral intrabony periodontal defects were selected. Thus, a total of twenty affected sites were chosen as noticed primarily on the radiographs and confirmed clinically as well as within the reconstructive surgical intervention. These defects were distributed into two groups as follows:-

i. Group 1 (the control group): Ten sites received flap debridement followed by the application of bioabsorbable equine collagen membrane* (Biocollagen®).

ii. Group 2 (the test group): Ten sites received flap debridement followed by the application of the same bioabsorbable collagen membrane but combined with equine bone graft material as xenograft† (Bio-Gen®).

Primary Assessment and Patient's Preparation

Patients were subjected to pre-surgical, clinical and radiographic interpretation. The patients completed a thorough plaque control regimen and a strict oral hygiene instruction. Full-mouth phase I therapy was done using periodontal Gracey curettes# and an ultrasonic apparatus$. The reevaluation period was determined according to each individual response, with an average period of about 6 weeks. Thereafter, a treatment plan was defined and an additional non-surgical therapy and/or dental extractions were done whenever needed. However, the surgical intervention were started when the subject's plaque and gingival index had achieved at least 20% levels, according to instructions of some previous reports [19-21].

Clinical Measurements

Plaque index (PI), gingival bleeding index (BI), gingival recession (GR); probing pocket depth (PPD) and clinical

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attachment level (CAL) were recorded. An acrylic stent with reference points was used to localize the measurement sites at baseline and 18 months postoperatively. The periodontal measurements were recorded using a graduated William's periodontal probe‡. Tooth mobility was also graded [22], scored and evaluated.

Surgical Procedures

Access to the defects were done using full-thickness flaps and sulcular incisions through the bottom of the crevice, extending mesial and distal to the adjacent teeth and including the flap papillae. No releasing incisions needed on either sides of the flap. Granulomatous tissues were curetted and thorough root planning was performed. EDTA with a concentration of 24%, at neutral pH, was used then washed with saline irrigation.

Randomly, using a coin, one defect treated with equine bioabsorbable collagen membrane as a control group whereas the other defect was treated with the same collagen membrane but combined with equine bone graft (the test group). Graft material was soaked with sterile saline and condensed gently in the defects to the adjacent crestal walls. The collagen membrane was adapted to obtain precise application to the interproximal area of the affected site. The membrane was then adjusted to completely cover the defect, overlapping at least 2-3 mm of the residual bone and sutured adequately with bioabsorbable sutures. Flaps were repositioned coronally during wound suturing, if required. The surgical site was covered with a non-eugenol periodontal dressing% on the buccal and lingual aspects.

Post Operative Care

Chlorhexidine digluconate mouth rinse (0.12%) was used as two times daily for one month postoperatively. Amoxicillin trihydrate 875 mg/clavulanic acid as potassium salt 125 mg (Hibiotic 1gm film coated tablets, Amoun Pharmaceutical Co. SAE., El-Obour city, Cairo, Egypt), was prescribed as two times daily for 10 days. Also, a non-steroidal anti-inflammatory drug (Ibuprofen 600 mg, "Brufen granules", effervescent sachets, Kahira Pharm. & Chem. Ind. Co. under license of Abbott Laboratories-USA) was also prescribed twice per day, for 7 days. In addition, anti-protozoal drug (Secnidazole 1gm, Nitroimidazole derivative, Cipazole forte, SIGMA Pharmaceutical Industries-Egypt, with cooperation of Queen Pharm international) was prescribed two tablets (each tablet contains Secnidazole 1gm) as a single dose (one day treatment) during meal.

Postoperative care was performed weekly in the first month, monthly up to six months and then every 3 months. In cases of membrane exposure, Doxycycline 100 mg was prescribed 2 times per one day then once for 5-7 days, and the surgical sites were carefully cleaned with a cotton swab soaked with 0.12% chlorhexidine digluconate two times daily.

Assessment of radiographic parameters and radiographic interpretation

Standardized intra-oral periapical radiographs (Kodak X-ray film, USA) were obtained at baseline and 18 months postoperatively. These radiographs were taken using long cone/extension cone paralleling technique with a positioning device mounted on a roentgen machine, operating at 70 Kilo Voltage Power.

Radiographs were scanned using a digital scanner at an input of 300 dpi and 100% scale, then they were analyzed using a software@. The images had 768 × 512 pixels and 256 gray scale level. The alignment of images, in the pairs of radiographs, was applied to correct small geometric misalignments. Gray level was then calibrated to indicate changes in the radiographic density (RD).

In addition, the following measurements were obtained in millimeters: distance from cementoenamel junction (CEJ) to base of the defect (BD) and from CEJ to alveolar crest (AC). The differences between baseline and 18-month postoperative values for CEJ–BD indicated the change in the radiographic defect depth (RDD), whereas the differences for CEJ–AC suggested the possible occurrence of crestal bone resorption (CBR).

Statistical Analysis

The present study had parametric variables. Thus, student's paired t-test was used to compare the changes in the data from baseline up to 18 months postoperatively within each treatment group. On the other hand, the intergroup comparison was accomplished by independent sample t-test. A 'P' value of 0.05 or less was considered statistically significant.

Results

Using student's paired t-test, the intra-group comparison showed comparable outcomes in the experimental groups regarding plaque index, gingival bleeding index as well as gingival recession, when comparing baseline scores to 18 months postoperative data. This is because there were no statistically significant differences noticed for these parameters, except only gingival recession within group 1 where P value recorded as 0.04. In addition, using independent sample t-test for intergroup comparison there were no statistically significant differences found between the two groups for these clinical parameters (PI, BI & GR). (Table 1).

On the other hand, by using student's paired t-test, statistically significant differences were found when comparison was done from baseline up to 18 month postoperatively within both groups (1 & 2) regarding PPD, CAL, RDD & RD. Furthermore, using independent sample t-test for intergroup comparison there were also statistically significant differences found between the two groups for these clinical and radiographic parameters (PPD,

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CAL, RDD & RD) (Table 1).

Table 1: Comparison of clinical and radiographic parameters within and among study groups.

Parameters	Groups	Group 1 (N=10)			Group 2 (N=10)			Student's paired t test (comparison within each group)				Independent sample t test (comparison between experimental groups)	
								Group 1		Group 2			
		M	SD	SEM	M	SD	SEM	P value	Sig.	P value	Sig.	P value	Sig.
PI	Baseline	1.20	.63	.20	1.20	.65	.21	.23	NS	.09	NS	1.00	NS
	18 Month	1.03	.36	.11	.93	.37	.12					.55	NS
BI	Baseline	1.23	.85	.27	1.20	.79	.25	.32	NS	.07	NS	.95	NS
	18 Month	1.10	.54	.17	.90	.43	.14					.37	NS
PPD	Baseline	7.80	1.75	.55	7.90	1.66	.53	.000	S	.000	S	0.90	NS
	18 Month	5.2	1.40	.44	4.1	.99	.31					.05	S
GR	Baseline	.80	.79	.25	.70	.82	.26	.04	S	.17	NS	.79	NS
	18 Month	1.20	.42	.13	.90	.74	.23					.28	NS
CAL	Baseline	8.6	1.26	.40	8.6	1.35	.43	.000	S	.000	S	.74	NS
	18 Month	6.4	1.26	.40	5.0	.94	.30					.01	S
RDD	Baseline	5.9	1.10	.35	5.9	.99	.31	.000	S	.000	S	1.00	NS
	18 Month	3.5	1.08	.34	2.6	.70	.22					.04	S
RD	Baseline	92.4	12.38	3.92	94.10	14.32	4.53	.000	S	.03	S	.78	NS
	18 Month	99.5	10.91	3.45	114	17.68	5.59					.04	S

Group1: bioabsorbable equine collagen membrane.

Group 2: bioabsorbable equine collagen membrane plus equine bone graft.

P Value: Statistically significant as ($P \leq 0.05$).

Sig: significance; NS: Statistically not significant; S: Statistically significant.

M: Mean; SD: Stand Deviation; SEM: Stand error mean.

PI: Plaque index; BI: Bleeding index; PPD: Probing pocket depth; GR: Gingival Recession; CAL: Clinical attachment level; RDD: Radiographic defect depth; RD: Radiographic Density.

The probing depth is reduced from (7.80±1.75 mm) at baseline to (5.20±1.40 mm) at 18 month with a mean difference of (2.60±1.57 mm) in group 1, whereas from (7.90±1.66 mm) to (4.10±.99 mm) with a mean difference of (3.80±1.33 mm) in group 2. This was statistically significant (P = 0.000). The intergroup comparison indicated that the difference was statistically significant with a P value of 0.05 (Table 1).

The clinical attachment level is changed from (8.60±1.26 mm) at baseline to (6.40±1.26 mm) at 18 month postoperative with a mean CAL gain of (2.20±1.26 mm) in group 1, whereas from (8.60±1.35 mm) to (5.00±.94 mm) with a mean CAL gain of (3.60±1.15 mm) in group 2. This was statistically significant (P = 0.000). The intergroup comparison indicated that the difference was statistically significant with a P value of 0.01 (Table 1).

The radiographic defect depth was reduced from (5.90±1.10 mm) at baseline to (3.50±1.08 mm) at 18 month postoperative with a mean difference of (2.40±1.09 mm) in group 1, whereas from (5.90±.99 mm) to (2.60±.70 mm) with a mean difference of (3.30±0.84 mm) in group 2. This was statistically significant (P = 0.000).

The radiographic density was changed from (92.40±12.38) at baseline to (99.50±10.91) at 18 month postoperative with a mean difference of (7.10±10.65) in group 1, whereas from (94.10±14.32) to (114±17.68) with a mean gain of (19.90±16.00) in group 2. This was statistically significant (P = 0.000 & 0.03 respectively). In addition, the intergroup comparison regarding both RDD and RD showed that the difference between the studied groups was statistically significant with a P value of 0.04 (Table 1).

Two cases are presented in figures from 2 to 5 with clinical photographs during surgical intervention and pre-& post-operative radiographic images. Case 1 is presented in figures (1 & 3) and was treated by using bio absorbable equine collagen membrane, for covering and treatment of selected intrabony defect site between teeth No. 26 & 27 as mainly distal to tooth No. 26. Case 2 is presented in (figures 4 & 5) and was treated by the application of the same bio absorbable collagen membrane but in combination with equine bone as xenograft material, for covering and treatment of selected intrabony defect site between teeth No. 35 & 36 as mainly mesial to tooth No. 36.

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Figure 1: Clinical photograph showing research materials used in the present study [bioabsorbable equine collagen membrane (Biocollagen®) & equine bone graft (Bio-Gen®)].

Figure 2: Clinical photographs of case 1 showing intrabony defect site (distal to tooth No. 26) after surgical reflection from both palatal (A) & buccal (B) sides and following application of bioabsorbable equine collagen membrane in both palatal (C) & buccal (D) sides.

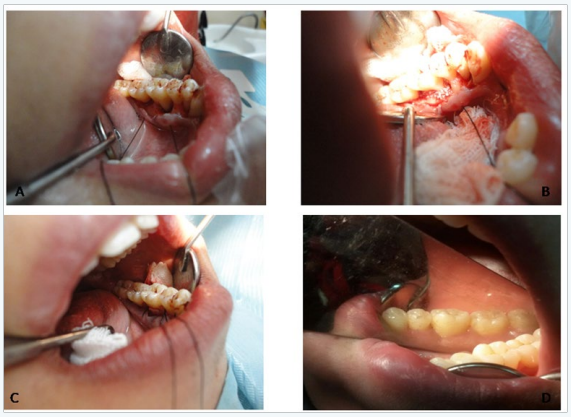

Figure 4: Clinical photographs of case 2 showing intrabony defect site (mesial to tooth No. 36) after surgical reflection of the flap (A), placement of equine bone graft particles soaked with saline (B), placement of bioabsorbable equine collagen membrane (C) & a postoperative follow-up photograph (D).

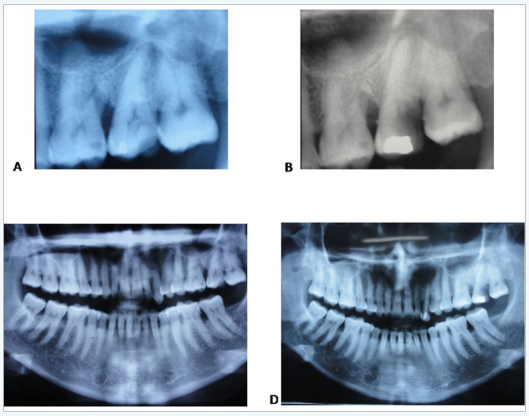

Figure 3: Radiographic images of case 1 showing pre-(A) & post-(B) operative periapical radiographic images as well as pre-(C) & post-(D) operative panoramic images.

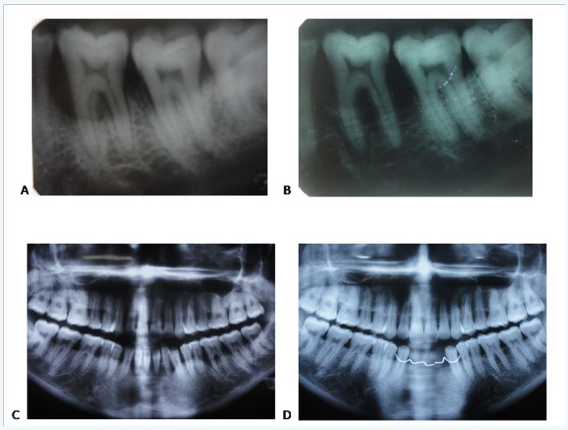

Figure 5: Radiographic images of case 2 showing pre-(A) & post-(B) operative periapical radiographic images as well as pre-(C) & post-(D) operative panoramic images.

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Discussion

It has been established in the previous literature [10,23] that the exclusion of epithelial and gingival connective tissue cells by using GTR barriers is important for periodontal wound healing, in order to achieve regeneration of the attachment apparatus. However, the non-restorable barriers have some disadvantages, such as higher cost, membrane exposure, need of a second intervention for their retrieval, complexity and bacterial accumulation [24,25]. Several restorable barriers are therefore developed to decrease such drawbacks. These are preferable and widely used for guided tissue and/or bone regeneration [26,27].

Collagen membranes are selected frequently as restorable barriers, especially they possess some advantageous properties. These are a low toxicity due to a low immune response, the ability of collagen to reconstitute into the natural tissues and to enhance cell growth and attachment [28,29]. In addition, collagen membranes are absorbed quickly to provide the needed integrity during regenerative process.

Bone grafts are used to treat different types of alveolar bone defects. They have a function to act with osteoconductive or osteoinductive properties. They can maintain a space and play an evident role by preventing membrane from collapse in the bone defect [30,31]. They can also support the flap, facilitate the wound stability process and enhance the regenerative procedure [32]. Equine bone graft showed a favorable clinical and histological outcomes and an effective therapy for periodontal guided tissue and bone regeneration of intrabony defects, especially when combined with equine resorbable membranes [17,18].

Regeneration of aggressive periodontal defects is considered as a real challenge. Some earlier studies [33-36,13] used different graft materials and barrier membranes, either alone or in combination, to achieve periodontal regeneration. However, the treatment outcome showed the combination therapy (GTR membrane + bone graft) as more effective than using GTR membrane or graft alone. Most of these studies showed a combination of GTR membrane with either allograft (DFDBA), xenograft (Bio-Oss), hydroxyapatite, or enamel matrix proteins. However, in recent years, some evidence [17,18] suggested that equine bone graft and equine membrane are also capable of supporting the periodontal regenerative healing capacity. The present study was therefore planned to evaluate and compare the efficacy of using equine bio absorbable barrier, either alone or combined with equine graft, in treating intrabony defects of aggressive periodontitis.

It has been noticed that the clinical measurements have a critical role in evaluating regenerative process since they can provide reliable information regarding probing depth reduction and clinical attachment level gain. The studied groups of the present study showed significant improvement of PPD & CAL parameters when comparing baseline with 18 month post-operative data (Table 1) & (Figure 6), thus signifying the regenerative role of GTR materials. In this context, Garrett in (1996) [37] stated that use of GTR membrane enhanced successfully re-growth of the destroyed periodontium; however, there are some variations in the clinical predictability, degree of efficacy, and even the histological outcomes as postulated by Bartold et al. [38]. In addition, the intergroup comparison showed more probing depth reduction and greater attachment level gain in group 2 (the combined therapy) over group 1 (GTR alone) (Table 1) & (Figure 6), since statistically significant differences were found at 18 months post-operatively. This means that the attachment gain complied with the findings obtained in the previous studies [33-36,12-14]. However, there was a statistically significant difference found within group 1 when comparing gingival recession parameter from baseline up to 18 month denoting some gingival recession increase occurred in this group, whereas no significant difference noticed within group 2. On the other hand, the comparison between the two groups did not notice significant difference for gingival recession (Table 1). In addition, the membrane exposure was occurred in only one defect site per each group, but controlled with a careful post-operative care without complication.

The radiographic analysis is one of the valid methods to demonstrate the effectiveness of regenerative therapies, since it plays a certain role in determining treatment outcome through offering a non-invasive method of evaluating the hard tissue response to therapy. The radiographic analysis of the present study included linear measurements and bone density assessment using gray level. The results showed significant defect resolutions through an evident improvement in defect depth as well as a gain in bone density in both studied groups, when comparing baseline data with results of 18 month. Also, the intergroup comparison showed statistically significant differences favoring the results of the combined therapy (GTR + graft) over using GTR alone (Table 1) & (Figure 6). On the other hand, the changes in alveolar crest height were comparable in both groups. These results are consistent with the obtained findings of some previous reports [34-36,13-16,39].

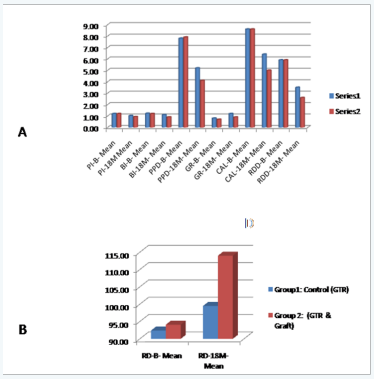

Figure 6: Histogram showing clinical & radiographic parameters among studied groups at baseline (B) and 18 month (M) postoperatively. A) includes these parameters: PI =Plaque Index, BI=Bleeding Index, PPD=Probing Pocket Depth, GR=Gingival Recession, CAL=Clinical Attachment Level and RDD= Radiographic Defect Depth. B) includes this parameter: RD= Radiographic Density.

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The significant improvement of the combined therapy in the present study may be explained by the use of bone graft as defect filler. In this context, some studies [14,40] suggested that treatment of periodontal intrabony defects with graft materials lead to significantly greater gain in clinical attachment level and better defect fill, by promoting osteogenesis and allowing rapid and quick formation of new bone. Also, it appears that the graft material has a critical role in preventing collapse of the membrane and/or flap during initial healing periods, thus can potentiate regeneration [32].

Regarding the positive findings obtained in the present study and as a significant point of view, it has been noticed that the ten involved cases were selected carefully with a complete patient desire to treat the affected defects, to strictly follow instructions to maintain oral hygiene and to attend needed follow up visits in due times with almost a complete compliance. The subjects had comparable bilateral intrabony defects. Their ages were below thirty in seven subjects and above thirty in only three patients. Mobility did not has any worsening in their grades throughout the whole study interval. Regarding pocket depth reduction, the number of defects that showed a successful resolution were four in Group 1 (GTR alone) and seven in group 2 (combined therapy), whereas the remaining defects (six in group 1 and three in group 2) still had a severe probing periodontal pocket depths. These remaining defects ranged in their depths from 5 to 8 mm in group 1 but from 5 to 6 mm in group 2. Regarding radiographic defect depth resolution and radiographic density gain, group 2 showed favored significant results, but no defects showed complete fill radiographically.

Conclusion

In final conclusion, although the present split-mouth clinical study had some given constraints, the combined therapy of using graft material (equine bone, Bio-Gen®) with GTR bio absorbable membrane (equine collagen, Biocollagen®) showed enhanced and significant clinical outcomes (PPD & CAL) over using GTR alone. Also, the radiographic assessment that evaluated defect fill (RDD) and bone density (RD) showed significantly greater results of the combined therapy. However, the studied groups showed significant improvement of these parameters when comparing baseline data with results of 18 month postoperatively within each group.

Meanwhile, we are planning to make further future long-term follow up (3 & 5 years) of results of the present study. However, further studies with larger sample size and different evaluation methods such as histological assessment may be recommended.

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Foot Notes

1. *Biocollagen®, the product support bone remodelling phase, Lyophilized Equine bioabsorbable collagen membrane, 25 x 25 x 0.2 mm, Bioteck, Torino, Italy.

2. †Bio-Gen®, bone tissue of animal Equine origin "xenograft", deantigenized for total reabsorption, Bio-Gen Mix, Cortical-Spongy, GR. 0.5 size 0.5 – 1 mm, Bioteck, Torino, Italy.

3. #Hu-Friedy, Chicago, IL, USA.

4. $Cavitron, Dentsply, NY, USA.

5. ‡Florida probe, Florida Probe Corporation, Gaineswille, FL, USA.

6. %Coe-pak, GC America.

7. @Emago Dental Software, Oral Diagnostics Dental Systems, Amsterdam, The Netherlands.

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