Section: Research Paper

Effectiveness of autogenous bone graft combined with various growth factors in angular bone defects

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Abstract:

Objective: The current study aims to investigate whether combined ABG and EMD treatment of angular bony defects in chronic periodontitis patients results in better clinical, radiographic, and biochemical outcomes than OFD treatment alone.

Methodology: This parallel, controlled, prospective, and randomised clinical trial compared two potential treatments for angular anomalies. ABG combined with EMD used to treat the faults in the test group whereas OFD was used to address those in the control group. Ten defects were assigned to each group because certain issues can go away during the follow-up period. With a mean age of 44.17 ± 7.80 years and 12 chronic periodontitis patients (6 men and 6 women) were reported, between September 2021 and September 2022, with 20 defects equally allocated to both group.

Results: All treatment modalities resulted in statistically significant improvements at six months (P< 0.01). The use of EMD—either with ABG or alone—was observed to induce significantly less recession than the OFD (P <0.05), while the combination and EMD groups demonstrated significantly better increases in attachment level (P< 0.01) and radiographic defect fill (P< 0.05) than the control group.

Conclusion: The findings show that there are no clinical or radiographic differences between the combination and EMD groups, but that EMD has a beneficial effect on the amount of GCF TGF-1, which rises during the healing period.

Keywords: Autogeneous bone graft, Angular bone graft, Growth factors

Introduction:

The ultimate goal of regenerative periodontal therapy is to prevent future attachment loss and to rebuild the supporting tissues that were damaged or destroyed due to disease or injury. [1] These tissues include the alveolar bone, periodontal ligament, and root cementum.[2,3,4,5] The commercially available enamel matrix derivative (EMD) product with autogenous bone graft (ABG) is a highly suggested regeneration treatment for intrabony defects due to its gel-like nature. [6] ABG is one of the greatest graft materials available today because of its osteogenic, osteoinductive, and osteoconductive properties. [7,8] Two distinct types of wound healing can be achieved in the intrabony defect as a result of the synergistic interactions between ABG and EMD.[8] While the ABG provides osteoinductive and/or osteoconductive effects, maintains space, stabilises the biomaterial, and inhibits flap collapse, the EMD can exert biological potential by encouraging the growth of new periodontal ligament and cementum. Numerous clinical studies examining the application of EMD + ABG in intrabony defects have produced outcomes that are equivalent in terms of parameters indicating attachment gain (AG) and bone fill.[8,9,10]

Although the underlying mechanism of EMD during the healing of periodontal wounds is unclear, some evidence from in vitro studies has been found. The usage of EMD has shown impacts on the attachment, proliferation, chemotaxis, spreading, and survival properties of many types of periodontal cells in addition to the expression of a number of chemicals, including extracellular matrix molecules, cytokines, and growth factors.[11] There is little information available about how EMD affects periodontal wound healing after nonsurgical or surgical therapy by measuring the level of any biomarker in gingival crevicular fluid (GCF). [12,13,14] GCF may serve as a proxy for ongoing periodontium-related processes like tissue creation, remodelling, inflammation, and destruction.[15,16,17,18,19] During the initial stages of wound healing, both active neutrophils and activated macrophages produce transforming growth factor-1 (TGF-β1), a multifunctional peptide.[16,17,18,19,20,21,22,23] It

demonstrates an essential role in the tissue remodelling and regeneration phases of wound healing by encouraging cell differentiation, proliferation, and expression.[24,25,26] TGF-β1 alone or in conjunction with other growth factors has the power to speed up various stages of wound healing.[24,26,27] It helps in the development of new granulation tissue by promoting angiogenesis and the production of collagen by fibroblasts.[16,22,28,29] One of the many clinical conditions where TGF-β1 controls collagen synthesis is periodontitis. It has been proven that TGF-β1 can partially induce periodontal regeneration in vivo.[16,30,31] The relationship between angular bone defects and trauma from occlusion is not exclusive since angular bone loss deficits are linked to teeth with normal function and in occlusion. The majority of the angular defects are due to the apical extension of the subgingival plaque. Osseous defects can exist alone or in a variety of arrangements. Finding osseous anomalies is crucial since osseous operations are diagnosis-based, which makes them clinically challenging. The complexity of the disease increases the relevance of using imaging techniques in the detection of such anomalies. Radiographs are essential for periodontal diagnosis because they can reveal the type and degree of damage done to the alveolar bone.The objective of the current study was to assess the clinical, radiographic, and GCF TGF-β1 levels of open flap debridement (OFD), ABG, and EMG applied to angular bone defects in chronic periodontitis patients. The current study aims to investigate whether combined ABG and EMD treatment of angular bony defects in chronic periodontitis patients results in better clinical, radiographic, and biochemical outcomes than OFD treatment alone.

Methodology:

This parallel, controlled, prospective, and randomised clinical trial compared two potential treatments for angular anomalies. ABG combined with EMD used to treat the faults in the test group whereas OFD was used to address those in the control group. With the exception of the use of grafts and the application of EMD to the root surfaces, both groups underwent the same periodontal flap procedure. Clinical and radiological features were evaluated before the surgery and six months later. The sample size was calculated by using recent clinical study and we considered 1.85 mm difference in AG score between the groups, a 1.35 mm standard deviation, and a 5% error with 80% power was clinically meaningful.[32] It seems that a sample of nine defects is needed for each category. Ten defects were assigned to each group because certain issues can go away during the follow-up period. With a mean age of 44.17 ± 7.80 years and 12 chronic periodontitis patients (6 men and 6 women) were reported, between September 2021 and September 2022, with 20 defects equally allocated to both group. Prior to the trial, each participant signed an informed consent form. The periodontology department of the faculty of dentistry provided patient care at its clinics.The following

conditions had to be met in order to be included: (a) absence of systemic diseases that would preclude periodontal surgery and could affect the outcomes of the therapy; (b) abstinence from smoking; (c) abstinence from drugs; (d) absence of pregnancy or lactation; (e) good oral hygiene (plaque index [PI] 1][33] and full mouth bleeding on probing (BOP) score 20% after initial periodontal treatment (IPT); (f) Patients were assigned to the therapy groups clockwise if they had two or more intrabony defects.[10,34,35,36]

Prior to and six months following surgery, the deepest region of the defect was scored using the same periodontal probe (UNC 15, Hu-Friedy, Chicago, IL, USA) for the PI,[33] gingival index (GI),[33] BOP, PD, relative attachment level (RAL), and recession scores. In the examination, a measurement between two markers was treated as a 0.5 mm increase. Assessments of probing were made down to the nearest mm. Using an adapted acrylic stent with reference holes, one calibrated examiner (OBA), who was not blinded to the surgical techniques, carried out the clinical evaluations at six sites on the tooth: vestibulary (mesial, mid, and distal), and oral (mesial, mid, and distal).

All intrabony defects were screened using an appropriate screening equipment (RWT Roentgenographic-equipment, Kentzler-Kaschner Dental GmbH, Germany) utilising routine periapical radiographs at baseline and six months later. A digital camera (Canon Powershot G10, Japan) was used to digitise radiographs, and photo editing software (ACD Photo Editor 3.1, ACD Systems Ltd., USA) was used to process the grayscale images at a resolution of 8 bits by 300 dpi. Two radiographs that were acquired from the same defect were altered to be the same size before evaluation using the software's cut and resizing features. The obtained images were then examined by using image analysis software (Image J 1.43u, Wayne Rasband, National Institute of Health, USA). The formula used to calculate the radiographic bone fill percentage was (1 - [A2/A1 (L1/L2)2] 100), where L1 is the linear distance between the mesial and distal cementoenamel junctions on the baseline radiograph, L2 is the linear distance between the mesial and distal cementoenamel junctions on the postoperative radiograph, and A1 represents the baseline area defined by the borders of the defect.The depth of the intrabony defect (IDD) was determined by measuring the distance between the top of the defect and the maximum coronal section of the alveolar bone. During surgery, the defect configuration (i.e., the number of walls) was also documented. Paper strips were used to collect GCF samples prior to surgery as well as 7, 14, 30, 90, and 180 days thereafter. Then, in line with the manufacturer's instructions, they were examined using an enzyme-linked immunosorbent assay and a commercial TGF-1 kit.

A standard automated gingival fluid measuring device (Periotron 8000, Smithtown, New York, USA) was used to quantify the volume of the gingival fluid after inserting

the strips into sterile tubes, and samples were then kept at 80°C.Each procedure was performed by the same operator (OBA). Scaling and root planing were carried out on all patients as part of IPT using both hand (Gracey, SG 3/4, 5/6, 7/8, 11/12, 13/14, Minifive, SAS 3/4, Hu-Friedy, USA) and ultrasonic (Cavitron® Bobcat Pro®, Dentsply International Inc., USA) tools. After eight weeks, each fault was assigned at random to a group. After applying a local anaesthetic, sulcular incisions were performed, full-thickness flaps were raised on the buccal and lingual surfaces, granulation tissues were eliminated, and the root surfaces were gently scaled and planed. The operation room was thoroughly cleaned to remove any traces of blood and saliva.The test group's exposed root surfaces received a two-minute application of 24% EDTA gel. After that, saline was used to rinse the surgical region. The root surfaces and intrabony defects received injections of EMD gel. The required amount of ABG was manually scraped off adjacent bone surfaces, mixed with the gel, and injected into the bone defects. The ABG was then covered with the second injection of EMD gel.

The patients also received naproxen sodium (550 mg tablet) twice daily for 7 days, amoxicillin + potassium clavulanate (1000 mg tablet) twice daily for 7 days, and mouthwash containing 0.12% chlorhexidine + benzydamine hydrochloride twice daily for 4 weeks. For the first four weeks following surgery, mechanical teeth cleaning was not authorised in the operating room. Sutures were removed 14 days after the operation. In order to do supra-gingival teeth cleaning and polishing, patients were only called back once every two weeks for the first two months, once every three weeks for the third month, and then once a month after that.

Statistical Analysis:

Statistical software (SPSS® 15.0 for Windows, Chicago, IL, USA) was used to analyse the data. Nonparametric tests were used since the data did not have a normal distribution. The Friedman test and the Wilcoxon test were used for intragroup repeated-measures and compared intragroup comparison, respectively. A post-hoc Mann-Whitney U-test with Bonferroni adjustments was performed at the $P < 0.017$ level. $P < 0.05$ was used as the cutoff point for statistical significance.

Results:

Random therapy was given to 12 patients with a total of 30 intraosseous defects. There were no dropouts after the study's end. No adverse consequences were noticed. There were small issues like bleeding and swelling throughout the early phases of healing after surgery. The groups' initial clinical parameters were comparable, as seen in Table 1. At the beginning, there were no discernible differences between the groups in any of the clinical criteria ($P > 0.05$). The defect distribution and configuration were comparable (Table 2). The intragroup comparisons of the mean PI, GI, BOP, PD, RAL,

and recession scores are shown in Table 3. With the exception of the control group, which showed statistically significant regression (P< 0.01) at 6 months with regard to its baseline value, all measured parameters showed statistically significant improvements in all groups (P< 0.05). Additionally, paired comparisons between the control and treatment groups as well as the control and combination groups indicated statistically significant differences (P 0.05), although no significant differences were found for the clinical and radiographic measures (P > 0.05).Table 4 displays the changes in GCF volume over the course of the inquiry. Over the course of 180 days, the GCF volume in the control group gradually declined below the initial level, starting with a slight increase on day 7. In the control group, the GCF volume had decreased at six months from a baseline value of 1.03 ±0.59 L to 0.51±0.31 L. This reduction was apparent but not statistically significant (P > 0.05). [Table 4]. After increasing across the 7th and 14th day evaluation periods (P<0.05), the baseline GCF volume in the therapy group decreased to 0.62 ±0.53 L after 6 months (P<0.05) [Table 4].

TGF-β1 was not detectable in 25% of the total GCF samples, 41% of the control group, or 6% of the treatment group [Table 6]. By day 7, the TGF-β1 concentration in the control group had increased and persisted above the initial value for the duration of the evaluation. TGF-β1 concentration in the treatment group decreased from a baseline value of 4.39± 3.57 ng/mL to 3.63 ±1.85 ng/mL at 6 months (P > 0.05), changing similarly to the EMD group. On days 7 and 14, TGF-β1 levels in the control group slightly increased before dropping below the starting point at 6 months. However, this change could not be statistically evaluated because of the unknown TGF-β1 content in GCF samples. TGF-β1 levels decreased similarly in the therapy group, going from 3.68±3.15 pg at baseline to 1.86 ±1.47 pg after six months. These changes did not meet the threshold for statistical significance (P > 0.05).

Table 1: Characteristics of clinical parameters

Parameters	Treatment group	Control	
Probing depth (mm)	8.3 ± 1.7	7.6 ± 1.64	0.63
Gingival index			
Interproximal	0.9 ± 0.21	0.9 ± 0.21	0.3
Full mouth	0.24 ± 0.09	0.31 ± 0.15	0.32
Plaque index			
Interproximal	0.65 ± 0.24	0.75 ± 0.26	010
Full mouth	0.23 ± 0.06	0.36 ± 0.27	0.12
Bleeding on probing			
Interproxiamal	55 ± 10.54	62.5 ± 17.68	0.52
Full mouth	8.29 ± 2.33	8.5 ± 2.03	0.59

Intrabony defect depth (mm)	6.4 ± 1.95	5.6 ± 1.64	0.28

Table 2: Characteristics of Intrabony defect

	Treatment	Control
Number of defect walls		
1 walled	1	1
1 to 2 walled	6	9
1 to 2 to 3 walled	3	-
Defect Localization		
Incisor/	5	3
Premolar	2	2
Molar	3	5

Table 3: Comparison of clinical and radiograph parameters

Parameters	Treatment group				Control				
	Baseline	6th month	Change	p value	Baseline	6th month	Change	p value	Overall p-value
Probing depth (mm)	7.93 ± 1.66	3.22 ± 0.41	4.71 ± 1.63	0.005	7.6 ± 1.51	3.2 ± 0.79	4.4 ± 1.17	0.004	
Recession (mm)	5.12 ± 1.91	6.28 ± 0.82	-1.16 ± 1.62	0.064	4.7 ± 1.7	7.3± 1.49	-2.70 ± 0.95	0.004	0.001
Gingival index (mm)	0.78 ± 0.24	0.27 ± 0.34	0.51 ± 0.33	0.01	0.9 ± 0.21	0.7 ± 0.22	0.2 ± 0.22	0.038	
Relative attachment level	13.06 ± 1.77	9.51 ± 0.84	3.55 ± 1.46	0.005	12.1 ± 2.13	10.5 ± 1.78	1.6 ± 0.7	0.004	0.001
Plaque index (mm)	0.55 ± 0.16	0.05 ± 0.16	0.5 ± 0.00	0.002	0.75 ± 0.26	0.35 ± 0.24	0.4 ± 0.21	0.005	
Bleeding on probing (%)	60.33 ± 17.29	12.62 ± 13.05	47.71 ± 18.49	0.005	62.5 ± 17.68	37.5 ± 17.68	25 ± 0.00	0.002	0.002
Radiographic bone fill (%)			64.56 ± 24.23				35.31 ± 20.56		0.003

Table 4: Changes in clinical parameters

	At day 0	At day 7	At day 14	After 1 month	After three month	After 6 months	p-value
TGF-β1 concentration (ng/ml)							
Control	1.15 ± 0.51	1.61 ± 1.05	4.31 ± 8.74	2.93 ± 3.08	4.06 ± 4.57	2.75 ± 2.53	
Treatment	4.39 ± 3.57	6.03 ± 7.26	4.92 ± 4.89	4.88 ± 5.04	3.71 ± 3.59	3.63 ± 1.85	0.388
p-value	0.13	0.019	0.09	0.54	0.95	0.56	
GCF volume							
Control	1.03 ± 0.59	1.26 ± 0.55	1.10 ± 0.63	0.83 ± 0.45	0.56 ± 0.48	0.51 ± 0.31	0.06
Treatment	1.04 ± 0.74	1.23 ± 0.72	1.16 ± 0.83	0.88 ± 0.61	0.38± 0.15	0.62 ± 0.53	0.028
p-value	0.8	0.9	0.99	0.96	0.041	0.724	
TGF-β1 amount							
Control	1.47 ± 0.77	2.28 ± 2.09	1.74 ± 1.22	1.4 ± 1.11	1.4 ± 1.52	0.29 ± 0.15	
Treatment	3.68 ± 3.15	5.89 ± 4.85	3.04 ± 1.82	3.44 ± 2.46	1.45 ± 1.24	1.86 ± 1.47	0.141
p-value	0.22	0.18	0.02	0.23	0.713	0.05	

Discussion:

The primary objective of this experiment was to evaluate the healing effects of ABG in intrabony periodontal anomalies in conjunction with EMD. The findings demonstrated that every treatment strategy significantly and statistically improved clinical and radiographic outcomes. The current study demonstrates that EMD combined with ABG promotes considerable advantages over OFD alone when applied to intrabony defects. In this study, gingival health was evaluated using GI and BOP measurements. The interproximal GI and BOP measurements showed statistically significant changes between the groups at six months ($P < 0.05$). The test group experienced significantly higher decreases in these metrics compared to the control group. However, there is no statistically significant difference between the EMD and combo groups ($P > 0.05$). These findings are in line with studies that examined the potential advantages of combining EMD and OFD and discovered that doing so led to statistically significant soft tissue restoration when compared to utilising OFD alone.[23,45,37,38] This may be due to the antibacterial and anti-inflammatory effects of PGA as well as the

decreased levels of matrix metalloproteinase in the EMD-treated areas.[39,40,41,42]
In both groups, there were equivalent and significant PD reductions at 6 months ($P< 0.05$) according to clinical assessments, with no group differences ($P > 0.05$). Our findings—which revealed a 4.71 mm reduction in the test group—were in line with previous clinical studies[8,9,10,43,44,45,46,47], as were the PD reductions reported in investigations which examined the prospective effects of utilising EMD in conjunction with ABG and graft materials in intrabony defects. Studies examining the effects of OFD on intrabony defects with baseline PD scores larger than 6 mm found PD reductions of 1.4 to 4.5 mm.[45,47,48,49,50,51,52] The findings of these investigations are supported by the results of the current trial, which showed comparable PD reductions in the control group with 7.60 mm mean initial PD. PD reductions and gingival recession scores should be evaluated simultaneously in order to evaluate the regenerative response to periodontal therapy.[53] At 6 months, clinical assessments revealed considerable AG in both groups ($P <0.01$).
The relative AGs for the control and test groups were 1.60 ±0.70 mm and 3.55± 1.46 mm. When compared to the control group, the AG attained by the treatment group was substantially higher ($P <0.01$). These evidences were supported by AG in our control group as 1.60 ±0.70 mm. When compared to OFD in the current investigation, surgical application of EMD in intrabony defects exhibited substantial AG. This outcome was consistent with a systematic review that examined the possible advantages of taking EMD along with OFD.[54]

A larger number of studies have been unable to find any substantial modifications in AG, despite the fact that the use of EMD in combination with a variety of bone transplants has produced notable AG results.[55,56,57,58,59,60,61] The mean AG findings for the therapy group were 3.56 ±1.46 mm, respectively.According to the radiographic examination, the radiographic bone fill % within the defect was 35.31± 20.56 for the control group and 64.56 ±24.23 for the treatment group, respectively. The radiographic findings of other studies [62,63,64,65] that provide radiographically identified more newly created hard tissue concurred with the radiographic findings of the ones that were acquired. 50% bone fill was shown by Crea et al. [63]1 year after EMD administration.
GCF is a tool that offers a minimally invasive access to the periodontium. Monitoring the GCF's contents can help identify compounds in tissues and cells that are not just obtained from the microbiota but also from the host's reaction. Evaluation of the various molecular levels found in the GCF may be useful as a predictive indicator of systemic and periodontal health, as well as wound healing activity.[16,27,18,66,67,68,69]

The present study determined the amount of TGF-β1 present in the GCF during the healing process after treating intrabony defects with two various treatment techniques. TGF-β1 levels and GCF volume both rose in the early phases of recovery before falling to the equivalent baseline levels. According to Kuru et al.,[16] TGF-β1 may be measurable in GCF and its level briefly rises after nonresorbable membrane-based regenerative periodontal surgery. In a recent study, Ribeiro et al.[14] applied OFD with and without EMD using a minimally invasive surgical approach, and they measured the levels of mediators, including TGF-β1, which is involved in GCF, after periodontal surgery. This study showed that TGF-β1 levels increased in both groups after 15 days and then decreased to baseline levels after 3 months, which is consistent with the findings in our study. There were no variations in TGF-β1 levels between the groups in that study, contrary to what we discovered. Studies have shown that in addition to its stimulatory effects on many types of periodontal cells, EMD also contains TGF-β1 or TGF-like molecules, bone morphogenic protein-like growth factor, and bone sialoprotein-like molecules.[69,70,71,72] These biochemical findings that we made while analysing the GCF samples provide support for this in vitro evidence. However, there was no discernible difference in the amount of TGF-β1 levels in GCF found in our investigation when ABG and EMD were both administered as treatments of periodontal angular defects.

Conclusion:

The findings show that there are no clinical or radiographic differences between the combination and EMD groups, but that EMD has a beneficial effect on the amount of GCF TGF-1, which rises during the healing period.

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