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Erythropoietin Gel as an adjunct to Xenograft in the Surgical Management of Intrabony Periodontal Defects: A Randomized Controlled Clinical Study

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Aim: The present trial aimed to compare the clinical as well as the radiographic efficacy of Erythropoietin (EPO) gel as an adjunct to xenograft versus xenograft alone in treating intrabony defects.
materials and Methods: This research was conducted at the Faculty of Dentistry, Ain Shams University, in the Department of Oral Medicine, Periodontology, and Oral Diagnosis. It was a randomized, double-blind, and controlled study. Twenty-six (stage III) periodontitis patients, with (three-wall) intrabony defects, were randomly divided (Thirteen in each group) into test (EPO gel + xenograft) and control (xenograft alone) groups. The primary outcome was evaluating the changes in the periodontal parameters; the plaque index (PI), sulcular bleeding index (SBI), probing depth (PD), and clinical attachment level (CAL) and wound healing by the Early Healing Index (EHI). The secondary outcome was evaluating the changes in the radiographic parameters including the defect fill (DF) and alveolar crest changes.
Results: After the first and second weeks, the test group demonstrated a significant EHI reduction than the control group. At six months, there was a significant gain in CAL and DF in both groups with a significantly lower CAL gain and DF in the test group compared to the control group. There was a significant PD reduction with no significant difference between both groups.
Conclusion: EPO gel as an adjunct to xenograft may accelerate soft tissue healing after surgical management of intrabony defects. However, it does not enhance the xenograft regenerative outcomes.

Keywords: Vertical bone loss, Periodontitis, Regeneration.

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Introduction

Periodontitis by definition is a chronic condition that involves multiple factors. It is caused by the buildup of microbial biofilm and involves various interactions between specific bacterial species, harmful immune responses of the host, and various environmental factors.[1] The periodontal intrabony defect is a common form of bone loss that occurs in periodontitis and may cause tooth loss if left untreated.[2]

The optimal treatment goal for intrabony defects is the absence of bleeding on probing and achieving shallow sulcus depth associated with periodontal regeneration.[3] Periodontal regeneration started with the proposal of barrier membranes which could preserve the space needed for both periodontal and bone regeneration.[4] However, there were so many drawbacks such as increasing the cost, risk of exposure, and extending the operation time. Thus, bone grafting without a barrier membrane has been proposed as an alternative treatment modality.[5]

Bone grafts prevent the epithelial cells from migrating into the defect without the need for a barrier membrane, especially in the contained three-wall intrabony defect.[6] A wide range of biomaterials have been introduced to be combined with bone grafts aiming to achieve periodontal regeneration, However, evidence for the most effective combination of such agents has not been proven yet.[7]

Xenografts are used widely in the treatment of periodontal defects as they stimulate bone formation by osteoconductive properties.[8] The most prominent advantage of xenografts is that augmented bone can maintain its volume owing to their slow resorption properties after their surgical application.[9] Moreover, the combination of xenograft with other biomaterials enhances its osteoinductive property besides its osteoconductive property.[10]

Erythropoietin (EPO) is a glycoprotein hormone endogenously secreted by kidneys in response to hypoxia stimulating the formation of red blood cells (RBCs) (i.e., erythropoiesis) in the bone marrow.[11] The interaction of EPO with EPO-R is involved in erythropoiesis as well as in different intracellular signaling cascades in a variety of nonhematopoietic organs and cells.[12,13]

It is believed that EPO could enhance and accelerate the process of healing through its angiogenic property, enhancement of oxygenation, its antiapoptotic property in addition to the recruitment of stem cells to the affected healing site. It also possesses regenerative power which is useful in the management of acute and chronic tissue damage.[14]

In dentistry, EPO was used on surgical palatal donor sites to enhance healing and epithelization.[15] Also, the periodontal ligament stem cells (PDL-SCs) showed osteogenic differentiation when treated with EPO.[16] In 2021, Aslroosta et al[17] reported that the use of EPO along with non-surgical periodontal therapy led to a significant decrease in gingival inflammation, as well as a reduction in PD and CAL.

As per our knowledge, no previous trials tested the local application of EPO gel along with surgical treatment for intrabony periodontal defects. Based on the aforementioned host modulatory, osteogenic, and angiogenic properties of EPO, this study was done to assess EPO as an adjunct to xenograft in treating intrabony periodontal defects.

Material and methods

Study design

This trial was conducted in a randomized, controlled, double-blind design with a 1:1 allocation ratio in the Oral Medicine, Periodontology, and Oral Diagnosis department of Ain Shams University's Faculty of Dentistry in Egypt

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between July 2022 and May 2023. The study aimed to evaluate the use of erythropoietin gel as an adjunct to xenograft for treating intrabony periodontal defects surgically. The primary outcome was evaluating the changes in the clinical periodontal parameters and wound healing. The secondary outcome was evaluating the changes in the radiographic parameters. Before initiating the investigation, the Research Ethical Committee of the Faculty of Dentistry at Ain Shams University approved the study (approved number: FDASU-Rec IM012206 date: 19/1/2022). The patients were provided with detailed information regarding the proposed procedure. All patients were allowed to withdraw at any time, along with information on anticipated complications and the entire treatment before giving their informed written consent. The current clinical trial has been approved by Clinical Trials and registered (registration number: NCT05360511).

Inclusion criteria

1. subjects of both genders between the ages of 20 and 50.
2. subjects diagnosed with stage III periodontitis.[18]
3. Target site criteria include pocket depth ≥6mm, CAL ≥3mm, and three-wall intrabony defect according to the preoperative cone beam computed tomography (CBCT). All these criteria were assessed after phase I therapy by six weeks.
4. Subjects free from any systemic diseases according to Burket's questionnaire.[19]
5. Commitment to attend therapy sessions and recall visits, as well as following the instructions for oral hygiene measures.
6. No history of using antibiotics or periodontal therapy at the test site in the previous six months.

Exclusion criteria

1. Smokers.[20]
2. Pregnant or lactating females.[21]
3. Vulnerable individuals as mentally retarded individuals and prisoners.

Sample size calculation

To conduct a statistical test of the null hypothesis that there is no difference between test groups, a power analysis was designed with careful consideration. The chosen alpha level was 0.05, with a beta of 0.2 resulting in a power of 80%. An effect size (d) of 1.20 was calculated based on the findings of a previous study conducted by Mamajiwala et al[22] Using G*Power version 3.1.9.7, the estimated sample size (n) was determined to be 24 cases in total (12 cases per group). We performed 13 in each group for dropouts (i.e. a total of 26 patients).

Randomization

Twenty-six eligible patients were randomly allocated according to randomization created by the computer by using (www.Randomizer.org) in a ratio of 1:1. The patients were allocated by another individual other than the principal investigator into two equal groups.

Group A (Test group): 13 patients underwent open flap debridement followed by applying EPO gel + particulate xenograft.

Group B (Control group): 13 patients underwent open flap debridement followed by applying particulate xenograft only.

The process of allocation concealment involved the use of opaque sealed envelopes that were numbered consecutively.

Pre-surgical preparation

Periodontitis (stage III) eligible patients according to the inclusion criteria underwent phase I periodontal therapy which included oral hygiene instructions and 0.12% chlorhexidine rinse (Hexitol, The Arab Drug Company (ADCO), Cairo, A.R.E),

supragingival and subgingival debridement using ultrasonic (NSK, Multifunction Ultrasonic Scaler, Kanuma, Japan) and hand instrumentations (Gracey curettes7/8, 9/10, 11/12, 13/14; Hu- Friedy®, Chicago. IL, USA).

The periodontal reassessment was performed after six weeks to assess the sites with persistent pocket depth ≥6mm and CAL ≥3mm which require surgical intervention and were included in the study.[23] At this time point, CBCT was done to confirm the morphology of the three-wall intrabony defect.

Clinical assessment

The clinical parameters of the target sites were measured using a UNC graduated probe (University of North Carolina 15, Hufriedy®, Chicago, IL, USA) both at six weeks after the conventional therapy (baseline) and six months after the surgery. The periodontal parameters included plaque index (PI)[24], sulcus bleeding index (SBI) [25], pocket depth(PD)[26] assessed from the gingival margin to the base of the pocket, and clinical attachment level (CAL) assessed from the cementoenamel junction (CEJ) to the base of the pocket [26]. The evaluation of soft tissue healing was done using the Early Healing Index (EHI)[27], at the first and second weeks after the surgery.

Radiographic assessment

Radiographic assessment of the defect was done using CBCT (iCAT Next Generation Cone Beam 3D System LLC, Hatifield, PA, USA) at baseline and six months postoperatively. The superimposition process was carried out by combining the baseline image with the image taken six months after the surgery. The superimposition was achieved using On-Demand software(OnDemand 3D, Cybermed Inc., Seoul, South Korea).

The following views were evaluated on CBCT:

Baseline CBCT: The axial and sagittal views were evaluated to assess the morphology of three-wall intrabony defect to be included in the study.

Baseline and postoperative CBCT: The sagittal views were evaluated to measure the distance from CEJ to base of the defect (CEJ-BD) as well as the distance from CEJ to alveolar crest (CEJ-AC) Figure (1).

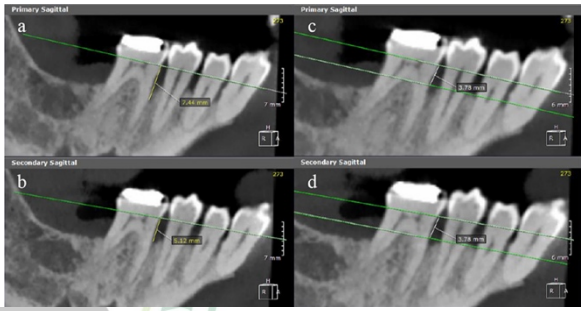

Figure (1): Representing the sagittal views at baseline and six months: (a & b) measurements of CEJ-BD and (c & d) measurements of CEJ-AC.

Preparation of carbon methyl cellulose (CMC)/ erythropoietin (EPO) gel [17]:

An alpha recombinant human erythropoietin liquid vial (Epoetin Alfa 4000 IU, SEDICO, Egyp) was used as a source of EPO and was transformed into a gel form. 800 mg CMC, 16 mg methyl-p-hydroxybenzoate sodium salt, 1.8 mg propylparaben is stirred with 4 ml sterile deionized water and was mixed using homogenizer at 10000 rpm for 5 min. Then, 13 ml of EPO vials were mixed with them using a homogenizer at 10000 rpm for 10 min. The final hydrogel with EPO was prepared and divided into 13 sterile syringes using a sensitive balance. Finally, syringes were sterilized using UV -waves. The preparation of the gel and all the above-mentioned steps were performed at Nawah Scientific Lab, Egypt. The syringes were kept wrapped in foil as EPO is sensitive to light and stored in the refrigerator at temperatures of 2°C to 8°C without freezing.

Particulate xenograft

Equine collagenated particulate graft with particle size: 250-1000 µm (OsteoBiol Gen-Os® by Tecnoss, Turin, Italy) was used in both groups.

Surgical procedures performed.

The operative site was anesthetized by local anesthesia (4% articaine and 1:200,000 epinephrine) (Artinibsa, local anesthesia, Inibsa Dental S.L.U. Spain). Sulcular incision was made using no. 15c blade (Tekno, sterile surgical blade, carbon steel, German). A full-thickness buccally and lingually/palatally flaps were reflected. Then, this was followed by removing the granulation tissue and debridement of the root surfaces in both groups. Then, the site was rinsed with sterile saline. In Group A (test group): The defect was filled with 0.25 cc of particulate xenograft mixed with 0.25 ml of EPO gel flushing with the alveolar crest (Figure. 2a-2d). In Group B (control group): The defect was filled with particulate xenograft alone flushing with the alveolar crest (Figure. 2e-2h). The flaps were then secured to their original position through a simple interrupted suture technique using 5-0 polypropylene sutures (D-tek polypropylene suture. Cyprus).

All the recruited patients received postoperative instructions, including 0.12% chlorhexidine hydrochloride rinse for two weeks. Patients' visits were scheduled after one week for removal of the sutures.

Statistical analysis

The study examined numerical data for normality using various tests, including the Kolmogorov-Smirnov and Shapiro-Wilk tests. The analysis showed that age, PD, CEJ-BD, and CEJ-AC had a normal distribution, while all other data had a non-normal distribution. The data was presented in different formats, such as median, range, mean, and standard deviation values. Depending on the distribution, various statistical tests were used to compare the two groups and study changes over time. For parametric data, Student's t-test and repeated measures ANOVA were used, along with Bonferroni's post-hoc test for pair-wise comparisons. For non-parametric data, the Mann-Whitney U test and Wilcoxon-signed rank test were used. The study used IBM SPSS Statistics for Windows, Version 23.0, based in Armonk, NY: IBM Corp. The significance level was determined at $P \leq 0.05$.

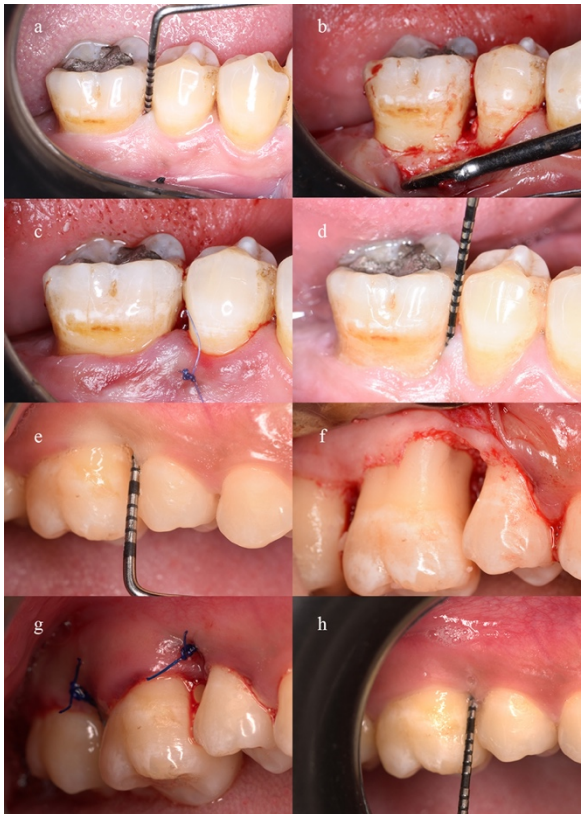

Figure (2): Representing clinical picture in the two groups. The test group (a) Pre-operative PD. (b) Application of the graft flushing with the alveolar crest. (c) Sutures securing the flaps. (d) six months post-operative PD. The control group. (e) Pre-operative PD. (f) Application of the graft flushing with the alveolar crest. (g) Sutures securing the flaps. (h) six months post-operative PD.

Results

Twenty-six patients were recruited, two patients did not complete the study and dropped out. Thus, the results and statistical analysis were done on 24 patients (i.e., 12 cases in each group). There were 3 (25%) males and 9 (75 %) females in the test group. While there were 2 (16.7%) males and 10 (83.3%) females in the control group. The mean (SD) age of the cases in the test group was 46.8 (6) years while in the control group, it was 40.3 (12.1) years. There was no significant difference between either group regarding gender (p=1) as well as age (p=0.105).

The intragroup comparison of EHI scores revealed a statistically significant decrease after two weeks in both groups. In addition, the intergroup comparison showed that after one as well as two weeks, the test group scores were statistically significantly lower than scores of the control group.

Table 1 Descriptive statistics and numerical data for comparison between EHI scores in the two groups and changes within each group.

Time	Test (n = 12)	Control (n = 12)	*P*-value	*Effect size (d)*
	Median (Range)	Median (Range)		
Week 1	2 (1, 3)	2.5 (2, 3)	0.011*	1.035
Week 2	1 (1, 2)	1.5 (1, 2)	0.028*	0.756
Percentage Change	50 (0, 50)	41.7 (33.3, 50)	0.844*	0.021
P-value	0.003*	0.001*		
Effect size (d)	3.464	4.152		

*: Significant at P ≤ 0.05

Regarding the SBI and PI, No significant difference was found in SBI scores of both groups for each observation period. Regarding the PI at six months, the test group showed a statistically significant increase. While for the control group, no significant change was found.

Table 2 Descriptive statistics and numerical data for comparison between SBI and PI in the two groups and the changes within each group.

SBI	Test (n = 12)	Control (n = 12)	*P*-value	*Effect size (d)*
	Median (Range)	Median (Range)		
Baseline	0 (0, 1)	0 (0, 1)	0.623	0.142
six months	0 (0, 1)	0 (0, 1)	0.623	0.142
P-value	0.655	0.655		
Effect size (d)	0.26	0.26		
PI	Test (n = 12)	Control (n = 12)	*P*-value	*Effect size (d)*
	Median (Range)	Median (Range)		
Baseline	0 (0, 0)	0 (0, 1)	0.317	0.142
six months	1 (0, 1)	0 (0, 1)	0.016*	0.937
P-value	0.003*	0.317		
Effect size (d)	3.464	0.603		

*: Significant at P ≤ 0.05

Regarding the changes in PD and CAL. Intragroup comparison showed significant differences from baseline to six months. Intergroup comparison of the percentage change of PD showed no statistically significant difference. For CAL, the test group showed a statistically significantly lower percentage decrease than the control group.

Table 3 Descriptive statistics and numerical data for comparison between PD measurements (mm) and CAL (mm) in the two groups and the changes within each group

PD	Test (n = 12)		Control (n = 12)		*P*-value	*Effect size (Partial Eta squared)*
	Mean	SD	Mean	SD		
Baseline	6.67	0.78	6.67	0.98	1	0
six months	3.67	1.23	3.33	0.98	0.472	0.024
Percentage change %	44.4	20.3	48.3	19.3	0.500	0.274
P-value	<0.001*		<0.001*			
Effect size (Partial Eta squared)	0.672		0.717			
CAL	Test (n = 12)		Control (n = 12)		*P*-value	*Effect size (d)*
	Median	Range	Median	Range		
Baseline	7	3, 9	5	3, 8	0.057	0.829
six months	4.5	3, 6	1.5	0, 7	0.009*	1.213
Percentage change %	42.9	0, 62.5	65	0, 100	0.023*	1.035
P-value	0,007*		0.003*			
Effect size (d)	2.486		3.31			

*: Significant at P ≤ 0.05

Regarding the changes in radiographic parameters; CEJ–BD and CEJ–AC from baseline to six months, the intragroup comparison showed a statistically significant decrease in CEJ–BD. Moreover, in the intergroup comparison of the percentage change of CEJ-BD, the test group showed statistically significantly lower values compared to the control group. The intragroup comparison of CEJ–AC revealed a non-significant difference at six months.

Also, the intergroup comparison of the percentage change was not statistically significant.

Table 4 Descriptive statistics and numerical data for comparison between CEJ-BD and CEJ-AC measurements (mm) in the two groups and the changes within each group.

CEJ-BD	Test (n = 12)		Control (n = 12)		*P*-value	*Effect size (Partial Eta squared)*
	Mean	SD	Mean	SD		
Baseline	6.81	1.19	6.38	1.34	0.408	0.031
six months	5.98	1.52	4.68	1.44	0.043*	0.173
Percentage change %	12.54	14.21	26.93	16.05	0.033*	0.969
P-value	0.007*		<0.001*			
Effect size (Partial Eta squared)	0.283		0.618			
CEJ-AC	Test (n = 12)		Control (n = 12)		*P*-value	*Effect size (Partial Eta squared)*
	Mean	SD	Mean	SD		
Baseline	3.87	1.09	2.33	0.63	<0.001*	0.449
six months	3.8	1.1	2.21	0.8	0.001*	0.425
Percentage change %	1.72	7.87	3.7	29.28	0.954	0.024
P-value	0.628		0.413			
Effect size (Partial Eta squared)	0.011		0.031			

*: Significant at P ≤ 0.05

Discussion

The biological agents have been widely used in promoting periodontal regeneration in adjunct to bone substitutes which serve for space maintenance. However, there is no evidence in the literature for the most effective combination of such agents.[7] This validates the search for new biomaterials. The Positive effects of EPO in angiogenesis, osteogenesis, and cell proliferation have been demonstrated in several studies.[14-16] In addition, xenografts are used widely in the treatment of periodontal defects owing to their osteoconductive properties[8]. Thus, the hypothesis of this trial was combining erythropoietin (EPO) gel with xenograft in the surgical management of intrabony periodontal defect will lead to superior outcomes than using xenograft alone.

Equine-derived particulate xenograft was applied in both groups, because of its osteoconductive property, biocompatibility, and slow rate of resorption.[28] Thus, preventing the collapse of the soft tissue inside the intrabony defect giving enough time for the regeneration to occur. In the test group, a carboxymethyl cellulose-based EPO gel was used as an adjunct to xenograft in the defect. Carboxymethyl cellulose was selected to be a carrier of the EPO owing to its hydrophilicity, bio adhesiveness, surface properties for cell adhesion, excellent biocompatibility and biodegradability.[29]

The clinical parameters were assessed using a UNC graduated probe and the radiographic changes were analyzed using CBCT imaging as it is beneficial and accurate in the diagnosis of intrabony defects by providing accurate information about the mesio-distal width as well as the facio-lingual dimensions of the intrabony defects without overlapping of structures.[30] Similarly, it is reliable in the assessment of the outcome of periodontal regenerative therapy.[31] Thus, a combination of radiographic findings with clinical parameters may be necessary to provide a more detailed view of periodontal regeneration in clinical studies.

This clinical trial revealed no statistically significant difference in the age and gender distribution between groups. In addition, the baseline clinical parameters revealed no statistically significant difference for all groups, which makes the treatment outcomes comparable at the follow-up.

Early surgical site healing was assessed using the early healing index (EHI) after the first and second weeks after the surgery. Both groups had a statistically significant decrease in EHI scores after two weeks. Moreover, after one and two weeks, the EPO group demonstrated statistically significantly lower EHI scores than the control group. EPO's effect on wound healing is likely to be mediated by enhancement of angiogenesis and accelerated wound epithelialization.[15] These results are in agreement with Fayazzadeh et al[32] who conducted a study on rats, where the effects of normal saline, EPO, and fibroblast growth

factor-2 (FGF-2) were compared to prevent skin flap necrosis. The group treated with EPO showed a smaller area of necrosis.

The intergroup comparison of the SBI showed no statistical difference in both the baseline and six month follow-up. Target sites were free of any gingival inflammation, ensuring an unbiased assessment of the study groups without any impact from inflammatory reactions that may have influenced the treatment outcomes. Regarding the intergroup comparison of the PI at six months, it showed statistically significantly higher scores in the test group than in the control group. Despite that, the sites of the test group were also free of inflammation as they were free of bleeding on probing. Plaque indices are inherently subjective and unavoidably imprecise, unlike the objectivity and accuracy of bleeding on probing as a clear indicator.[33]

Clinical assessment of both groups showed a significant reduction in PD and CAL measurements at six months. This can be explained by good quality of debridement through access open flap and use of xenograft for space provision which are essential factors in achieving periodontal regeneration in intrabony defects and have a great impact on the clinical findings irrespective of the adjunctive treatment procedures. This was in accordance with a systematic review that stated that the intrabony defects treated with bone grafts yielded better CAL gain in comparison to intrabony defects treated only with open flap debridement procedures.[8]

Regarding the intergroup comparison of the percentage change of CAL, the test group showed a statistically significantly lower percentage decrease than the control group (P-value = 0.023). This could be justified by the fact that EPO could have stimulated the process of angiogenesis and induced very high levels of VEGF which can jeopardize bone regeneration. As excessive VEGF may recruit large numbers of osteoclasts, resulting in the associated graft being resorbed, since VEGF enhances the differentiation and migration of osteoclasts.[34] Thus, early loss of the graft may have caused the test group to show less CAL gain.

Helmrich et al[35] demonstrated that when modified stem cells derived from human bone marrow were loaded onto an osteogenic graft to express rat VEGF. Eight weeks after implantation, the vascular density of the test sites was 3-fold greater than with control sites. However, there was a reduction in bone quantity in the test sites. They concluded that over-expression of VEGF can hinder the formation of bone tissue by causing an increase in the recruitment of osteoclasts and promoting bone resorption.

The result of our study was similar to Kao et al[36] who compared the use of BMP-2 + xenograft (test group) versus xenograft alone (control group) in grafting maxillary sinuses and demonstrated superior outcomes of the control group which was justified by the effect of an increase in osteoclast differentiation caused by the release of BMP-2 causing faster graft resorption.

Regarding the percent change in PD, no significant difference was found between the study groups. Probing depth donates inflammatory state changes rather than regeneration.[37] In addition, both groups reached similar values at six months very close to normal sulcus depth.

Regarding the intragroup comparison of the distance of CEJ-BD, there was a statistically significant decrease in both groups at six months with (P-value = 0.007) in the test group and (P-value < 0.001) in the control group. This means that the defect fill was significant in both groups. This can be interpreted by the inherent potential of the three-wall intrabony defects for periodontal regeneration as they provide a spatial configuration for blood clot stabilization.[38] Moreover, the application of xenograft after

complete debridement of three-wall intrabony defects can result in significant bone fill.[9]

The intergroup comparison of the percent change of the CEJ-BD showed statistically significantly lower values of the test group in comparison to the control group (P-value = 0.024) and (P-value = 0.033), respectively. This means that the defect fill in the control group was greater than the test group which was consistent and in harmony with the results of the CAL. This coincides with Hoidal et al[39] Who concluded that there was significant defect fill in both the test group (DFDBA+EMD) and control group (DFDBA) with superior CAL gain and defect fill in the control group at six months.

Regarding the alveolar crest changes (ACC) there was no significant crestal bone loss in both groups. As after the regeneration of periodontal defects, the bony changes affect mainly the inner component of the defect while resorption of the alveolar crest may be very limited or may not happen at all.[40]

This clinical research has some limitations such as (i) The evaluation of EPO release profile was not performed. (ii) Histological assessment would have given accurate information about the restored tissues, but it was not employed in the current study to avoid the risk of sacrificing the tooth. Thus, future experimental animal studies that allow histological assessment of regenerative outcomes after EPO use should be conducted.

Conclusion

EPO gel as an adjunct to xenograft may accelerate soft tissue healing after surgical management of intrabony periodontal defects. However, the addition of EPO to particulate xenograft does not appear to significantly enhance the xenograft regenerative outcomes.

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