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EVALUATION OF HYDROXYAPATITE AND HYDROXYAPATITE COMBINED WITH PLATELET-RICH PLASMA GRAFT IN TREATMENT OF INTRABONY PERIODONTAL DEFECT - A COMPARATIVE STUDY

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Hydroxyapatite bone graft, Platelet rich plasma, Intrabony periodontal defect and periodontal regeneration

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ABSTRACT

Introduction: periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by specific micro-organisms or a group of specific micro-organisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both. **Aim of the study:** The present study aimed to assess the effect of hydroxyapatite and hydroxyapatite combined with platelet-rich plasma graft in treatment of intrabony periodontal defects. **Subjects and Methods:** 24 periodontitis patients with intra-bony periodontal defects participated in this clinical trial and treated with full thickness mucoperiosteal flap surgical technique and Hydroxyapatite bone graft (goup I) or full thickness mucoperiosteal flap surgical technique and Hydroxyapatite bone graft combined with platelet-rich plasma graft (group II). Clinical outcomes included plaque index (PI), gingival index (GI), probing depth (PD) and clinical attachment level (CAL). Bone defect area was measured by digital periapical radiographs and bone density was calculated by Digora program. Measurements at baseline, 3-months and 6-months postoperatively were subjected to statistical analysis. **Results**: Both of hydroxyapatite bone graft (HA group) and hydroxyapatite bone graft with platelet-rich plasma (HA+ PRP group) resulted in statistically significant improvement in all clinical and radiographic parameters from baseline to 3-months and 6-months. But no significant difference was observed between both groups. After 3-months, HA group showed 5.05±.37 PD reduction and 6.65±1.11 CAL gain, while HA+PRP group showed 4.95±.96 PD reduction and 6.30±0.86 CAL gain. After 6-months HA group showed 3.50±.62 PD reduction and 4.60±1.17 CAL gain, while HA+PRP group showed 3.35±.67 PD reduction and 4.70±0.67 CAL gain. **Conclusion**: Both hydroxyapatite bone graft group and hydroxyapatite bone graft combined with platelet-rich plasma group showed significant reduction in clinical and radiographic outcomes after 6 months with no statistically significant difference between them**.** Both treatment modalities are successful procedures for treating intrabony defects

INTRODUCTION

Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by specific micro-organisms or a group of specific micro-organisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both[(1)]. Amongst various periodontal treatment modalities, grafting of biomaterials/bone substitute have been successfully used to accomplish

the reconstruction of lost attachment apparatus in deep intra-osseous defects[2]. Calcium phosphate ceramics have been widely used as bone graft substitute for treating periodontal intrabony defects and have produced clinically significant results [3]. HA biomateials are complex calcium phosphates which resemble bone mineral in their chemical composition {Ca10(PO4)6(OH)2} and has calcium-to-phosphate ratio of 1.67 [4]. Platelet-rich plasma (PRP) contains high concentrations of platelet and natural fibrinogen to a lesser extent. The properties of PRP are based on the production and release of several growth factors due to the activation of platelets [5]. Platelet rich plasma is used alone or in combination with bone graft materials to regenerate hard tissue in treatment of bone defects around immediate implants or treatment of defects due to peri-implantitis [6]. Del Fabrro, et al., [7]. in a review study and meta-analysis evaluated the role of PRP in surgical periodontal treatments. The results showed that using PRP along with graft materials may have positive effects on the results of treatment of periodontal bone lesions. Gouri et al., [8]. reported that there was a reduction in mean plaque and gingival index scores from baseline to 6 months in control group (hydroxyapatite bone graft) and experimental group (hydroxyapatite bone graft and platelet rich plasma graft) with no statistical difference between the groups at all time periods. Geeti [9]. reported that PRP/HA group presented superior result regarding PD reduction, CAL and radiographic bone fill than HA group. Camargo et al.[10], concluded that PRP treatment provided no statistically significant resolution of intrabony bony defects. From the differences and contradictions between the results and conclusions of the studies described above concerning clinical parameters (plaque index, gingival index, clinical attachment level, pocket depth) and radiographic evaluation, we aimed to carry out this study to contribute to clearing this dilemma by studying the effect of hydroxyapatite bone graft and hydroxyapatite bone graft combined with platelet rich plasma graft in treatment of intra-bony periodontal defects.

MATERIALS AND METHODS

Study design

This study was designed as a non-randomized clinical trial comparing the clinical and radiographic outcomes of Hydroxyapatite graft with full thickness mucoperiosteal flap surgical technique versus Hydroxyapatite graft combined with platelet-rich plasma graft with full thickness mucoperiosteal flap surgical technique in management of periodontal intrabony defects.

Study population

This clinical trial included 24 patients (14 females and 10 males, aged 20 to 45 years) with mean age (34 years old) suffering from chronic periodontitis. Recruitement of subjects was done from the outpatient clinic, Department of Oral Medicine and Periodontology, Faculty of Dentistry, Suez Canal University diagnosed with chronic periodontitis with at least one site with probing pocket depth (PPD) ≥ 5 mm, intrabony defects seen in periapical radiographs. Exclusion criteria included: 1) teeth with suprabony defects or 1-wall intrabony defects; 2) pregnant or lactating women; 3) taking any medication 3 months prior to the study; 4) patients who received any periodontal treatment 6 months prior to study initiation; 5) former or current smokers.

Pretreatment phase

Full mouth probing and radiographic examination was performed for the recruited patients at the initial examination phase. Proper motivation and education were offered to the patients to perform oral hygiene measures as twice daily tooth brushing

with soft toothbrush using modified bass brushing technique and once daily interdental cleaning with dental floss and interdental brushes for wide interproximal embrasure spaces. Full mouth supra and subgingival debridement was performed using ultrasonic device with supragingival scaling inserts followed by universal and Gracey's curettes for proper subgingival debridement. Patient preparation was completed over 2-3 visits, in two weeks. Local anesthesia was used when necessary for patient comfort. 0.12 % chlorhexidine HCL mouth rinse was prescribed twice daily for 2 weeks. Re-evaluation of the recruited patients was done after 4-6 weeks from the initial periodontal therapy to confirm the need for periodontal surgery. The criteria that indicated the necessity of surgical intervention included persistence of interproximal site with PPD ≥ 5 mm, CAL ≥ 4 mm, and interproximal intra-bony defects assessed by periapical radiographs.

Preparation of platelet-rich plasma graft (PRP):

Half an hour before surgery, 10.0 ml of blood was drawn from each patient by venipuncture of the antecubital vein. The blood was mixed with 1 ml anticoagulant citrate dextrose-A [ACD-A] and centrifuged at 5, 600 rpm to separate the platelet poor plasma from the erythrocytes, platelets and leukocytes. The centrifuge speed then is slowed to 2400 rpm to allow for further separation of the platelets and leukocytes from the red blood cells pack. Removal of this red blood cells pack yields 3.0 ml of plasma with concentrated platelets. The resultant platelet-rich plasma was stored at room temperature. Immediately before application the PRP was activated by clot initiator (100 IU of lyophilized human thrombin with 1 ml of 10% CaCl2 solution). Within a few seconds, the PRP preparation assumed a sticky gel consistency, the coagulated PRP + HA bone graft was placed up to the vertical height of the corresponding adjacent bone level. Surgical flaps were repositioned to the presurgical level and sutured with 3-0 silk suture utilizing an interdental, direct suturing technique achieving primary closure. Care was taken not to displace the graft material during suturing. A periodontal dressing (Coe-pack) was placed on the surgical area.

Surgical protocol:

After the pretreatment phase and re-evaluation, patients were recalled and allocated to receive either Hydroxyapatite bone graft HA (group I) or Hdroxyapatite bone graft with platelet rich plasma graft HA+PRP (group II). Under local anesthesia an intrasulcular incision was performed with #15c blade elevating a full- thickness mucoperiosteal flap. The access flap was raised buccally and lingually to expose 2-3 mm of the alveolar crest and thorough debridement was performed using area-specific curettes (Hu-Friedy, USA). After exposing the intrabony defect, it was evaluated to confirm that it was a 2- or 3-wall defect with ≥3mm depth. Group I received meticulous debridement and root planning of the intrabony defect area to remove all the inflammatory granulation tissue and HA bone graft substitute was used to fill the defect by gradual placement of incremental fragments till the defect space was completely filled. While group II received the same surgical technique, and HA+PRP graft was used to fill the defect avoiding any air voids from incorporating the grafted material. Bone graft substitute (HA) was placed up to the level of the alveolar bone crest. Then the mucoperiosteal flap was returned back to its position in both groups and sutured with 4-0 polypropylene non-absorbable interrupted sutures.

Postsurgical instructions: Postoperatively, all patients were instructed to take systemic antibiotics (Amoxicillin 500mg t.d.s) orally for 5 days to prevent postsurgical infection. Also, oral analgesics and anti-inflammatory drugs (Ibuprofen 600mg t.d.s) were prescribed to the patients for the first 3 days then whenever needed. Patients were

instructed to avoid any hard brushing and trauma to the surgical site for one week and to rinse with antiseptic mouth rinse (0.12 % chlorhexidine HCL) twice daily for 1 minute after brushing for a period of two weeks. Sutures were removed ten days after the surgery and patients were instructed to continue mechanical tooth cleaning of the treated sites using an ultra-soft toothbrush and roll technique for one month in addition to chlorhexidine twice daily. Then, patients were instructed to return to the regular brushing using a soft toothbrush. All participants were followed up at 3 months and 6 months post-surgically, where supragingival scaling was performed reinforcing the oral hygiene measures at each month. Clinical periodontal parameters. Clinical periodontal parameters were measured for all participants immediately before the surgery (baseline), 3months and 6 months post-surgically. Those clinical parameters included plaque index (PI) (Silness and Löe,)[11] and gingival index (GI) (Löe,) [12], probing depth (PD), clinical attachment loss (CAL). PD was recorded from the free-gingival margin till the base of the periodontal pocket and CAL from cemento-enamel junction (CEJ) to the base of the pocket using light force. Measurements were performed using William's graduated periodontal probe and were rounded to the nearest millimeter. Prefabricated occlusal acrylic stents were used to standardize the clinical measurements through guiding the periodontal probe to be inserted in vertical grooves within the stent parallel to the long axis of the tooth. Radiographic measurement: An intra-oral periapical digital radiograph was taken for each selected site using standardized long cone paralleling technique, at baseline, at 3 and 6 months post-surgery. All radiographs were digitalized using an optical scanner and transferred to the computer as JPEG image. Intra oral parallel periapical radiographic procedure: Direct standardized digital radiographs were achieved using VISTA Ray charged coupled device (CCD) System. The VistaRay CCD is a digital mini-x-ray system taking X-ray directly at the chair side with immediate image display. The CCD sensor size dimensions were with: 27.4x 39.0x6.3 mm (W x H x D mm) and it has an active surface area of 20x30 mm. Pixel size is 19x19 microns with a total pixel number of 1, 659, 000 pixels. The parallel periapical technique was performed using the rinn (XCP) periapical film holder and a long cone (sixteen inch in length) which was mounted to the X-ray tube and the plastic aiming ring of XCP film holder was fixed flush- ended with the round end of the long cone. The sensor was exposed toX--ray Fona intra oral X-ray machine using exposure parameters: 70 kilovolt and 6 milliampere for 0.06 seconds. The central ray was directed perpendicular to the sensor. The exposure parameters were considered fixed for all patients. After the exposure was terminated, the readout started automatically and the image was displayed gradually on the computer screen. When the read out was completed, the newly read image was automatically stored on the active patient card. Radiodensitometric analysis of the radiographs: it was carried out using the software of the Digora (2.5) system. The software analyzes the images through image restoration then image enhancement. Image enhancement technique allows density and contrast adjustment of the images for optimum visualization. Density measurements were calibrated by quantifying the image on 256 grey scales. Zero scale was given to the totally black regions (totally radiolucent), while 255 was given to the totally white areas (totally radio-opaque). The bone density (BD) at the affected area was recorded as follows: a rectangular area was drawn interdentally at the affected site (the dimensions of that area were standardized for each patient from the baseline to the last follow up visit) and the mean grey level was recorded.

Statistical Analysis

Data were presented as mean and standard

deviation (SD). Student's t-test was used to compare between the two groups. Repeated measures ANOVA test was used to compare between the two groups as well as to study the changes within each group. Bonferroni's post-hoc test was used for pair-wise comparisons when ANOVA test is significant.

RESULTS

The clinical periodontal parameters:

The clinical and radiographic parameters recorded for group I and group II throughout the study period are shown in table (1). The results of this clinical trial showed a statistically significant reduction in GI after 3months and 6 months in both groups but with no statistically significant difference between both groups at baseline as well as after three and six months. Similarly, there was a statistically significant reduction in PI scores after 3 and 6 months in group I and group II, but also with no statistically significant difference between both groups at baseline and after 3 and 6 months. After 6 months, a significant improvement was observed in PD values from baseline in both groups. Similarly, there was a statistically significant gain in CAL values from baseline to three and six months in both groups but with no statistically significant difference between both groups as shown in table (1). As regards the reduction in PD; there was a statistically significant reduction of PD from baseline to 6 months in each group.

Table (1): *Show clinical periodontal & radiographic parameters (mean ±SD) in both studied groups throughout the experimental period.*

		Group I		Group II	Post-hoc	P-value
		Mean±SD	Post-hoc	Mean±SD		
PI	Baseline	1.09± 0.46		1.16±0.43		0.93
	3months	0.64±0.30	P1<0.001**	0.68 0.38	P1<0.001**	0.96
	6months	0.32±0.18	P2<0.001**P3<0.001**	0.33± 0.25	P2=<0.001**P3=<0.001**	0.97
GI	Baseline	1.17±0.56		1.36±0.48		0.41
	3months	0.72±0.40	P1<0.001**	0.73±0.26	P1<0.001**	0.55
	6months	0.35±0.28	P2<0.001**P3<0.001**	0.33±0.13	P2<0.001** P3<0.001**	0.85
PD	Baseline	8.00±0.85		8.10±0.97		0.32
	3months	5.05±0.37	P1<0.001**	4.95±0.96	P1<0.001**	0.36
	6months	3.50±0.62	P2<0.001**P3<0.001**	3.35±0.67	P2<0.001** P3<0.001**	0.85
CAL	Baseline	8.00±0.85		8.10±0.97		0.77
	3months	5.05±0.32	P1<0.001**	4.95±0.96	P1<0.001**	0.24
	6months	3.50±0.62	P2<0.001**P3<0.001**	3.35±0.67	P2<0.001** P3<0.001**	0.93
BD	Baseline	126.49±1.58		127.29±1.29		0.13
	3months	127.08±0.91	**P1=**0.94	127.89±1.14	**P1=**0.08	0.23
	6month	133.07±1.00	**P2**<0.001**P3**<0.001**	134.42±1.73	**P2**<0.001** **P3**<0.001**	0.07

*Data expressed as mean±SD. P: Probability. *: significant (P<0.05).**: highly significant (P<0.001). Test used: Repeated measures ANOVA followed by post-hoc Bonferroni.*

P1: significance of baseline vs 3 months. P2: significance of baseline vs 6 months.

P3: significance of 3 months vs 6 months.

Radiographic parameters:

There was no significant difference between the two groups at baseline. Also at 3 and 6 months; there was no significant difference between the two groups. Within group I and group II, 3 months showed no significance difference compared to baseline time while 6 months showed significant increase in BD compared to baseline time. Also 6 months showed significant increase in BD compared to 3 months' time.

DISCUSSION

Periodontitis is an inflammatory disease that leads to degradation of periodontal tissues, causing tooth movement and eventually tooth loss [13]. In controlled clinical studies, grafting of intrabony periodontal lesions with HA resulted in an attachment level gain which was greater as compared with non-grafted surgically debrided controls [14]. PRP contains growth factors that influence wound healing and enhancing soft tissue healing and bone regeneration [15]. In the present study on comparing PI and GI scores within goup I; there was statistical high significant decrease of PI and GI mean scores at each of 3 months and 6 months when comparing each of them with PI and GI mean score at baseline, similar finding of the mean PI scores was reported by Yukna et al., [16]. Meffert et al., [117]. Mohammad et al., [18] who found statistically significant reduction in the mean PI and GI scores when compared PI and GI at three months and at six months with baseline. Within Group II, there was statistical high significant decrease of PI and GI mean scores at each of 3 months and 6 months when comparing each of them with PI and GI mean score at baseline, similarly, Gouri et al., [8] reported a reduction in mean of PI and GI scores at 6 months compared with baseline in both (PRP with HA) and (HA) groups. These observations suggest that HA was well tolerated in the soft tissues and does not seem to evoke any inflammatory response significantly [19]. On Comparing PD and CAL mean scores at the different visits within each group within group I; there was statistically highly significant decrease of PD and CAL mean scores at each of 3 months and 6 months when comparing each of them with PD and CAL mean score at baseline. This reduction of PD and CAL mean scores may be due to the healing of connective tissue that do encapsulation of the graft with a long junctional epithelium [20]. Similar observation was reported by Mohammad et al [18]. Within group II, there was statistical high significant decrease of PD and CAL mean scores at each of 3 months and 6 months respectively when comparing each of them with PD and CAL mean score at baseline. This may be due to beneficial therapeutic effects of PRP on hard and soft tissue healing, due to the contents of growth factors stored in the platelets [21]. Similar results reported by Gouri et al [8]. In the current study, on comparing bone density (BD) mean scores between the two studied groups at baseline, at 3 and 6 months, there was no statistically significant difference of BD mean scores between them. Comparison of mean of BD between the two groups in the baseline, 3 months, and 6 months visits. Within group I and group II, of the present study, 3 months showed no significance difference compared with baseline time while 6 months showed significant increase in BD compared with baseline time. Also 6 months showed significant increase in BD compared with 3 months' time. Mustafa et al., [22]. reported that no significant difference in radiographic assessment among group (HA) and group (HA+PRP) at 1st and 6th month intervals, while 3 months post-operatively the amount of radiographic density at the PRP side was significantly higher. This may be due to combination of HA with PRP promote bone formation, especially at early stages of bone healing[23].

CONCLUSION

From the present study, it can be concluded that, Hydroxy apatite bone graft and hydroxyapatite bone graft with platelet rich plasma are satisfactory regenerative materials that can be applied to fill intraosseous periodontal defect and led to improvement of clinical and radiographic parameters over the course of the study.

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