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CLINICAL EVALUATION OF ADVANCED PLATELET RICH FIBRIN COMBINED WITH NANO-CRYSTALLINE HYDROXYAPATITE BONE SUBSTITUTE FOR MANAGEMENT OF MANDIBULAR MOLAR GRADE II FURCATION DEFECTS: A RANDOMIZED CONTROLLED CLINICAL TRIAL

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ABSTRACT

Background: The current randomized controlled trial assessed for the effect of advanced platelet rich fibrin (A-PRF) + nano-crystalline hydroxyapatite bone substitute (n-HA) with open flap debridement (OFD) versus OFD alone in the management of mandibular molar grade II defects.

Methods twenty-eight sites in twenty-four patients with mandibular class II furcation involvement were selected and randomized into test (A-PRF+ n-HA+ OFD; n=14) and control (OFD; n=14) groups. All were assessed for clinical outcomes such as probing pocket depth (PPD), vertical clinical attachment level (VCAL) (primary outcome), horizontal clinical attachment level (HCAL), gingival recession and post-operative pain.

Results: A Statistically significant gain was in VCAL gain at 3,6,9 months and significant PPD reduction at 3 and 6 months were recorded in favor of the test group, while there was no significant difference between the two groups in HCAL at 3,6 and 9 months. Both groups show statistically significant reduction in post-operative pain after 7 days.

Conclusion: (A-PRF+n-HA+OFD) or OFD were effective in treating class II furcation involvement in mandibular molar teeth with respect to PPD reduction, VCAL gain and HCAL. The test group showed more significant reduction changes in PPD and VCAL gain compared to control group. Both groups showed statistically significant reduction in post-operative pain as measured with VAS score.

KEYWORDS: open flap debridement; furcation involvement; clinical attachment level ; guided tissue regeneration

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INTRODUCTION

Multi-rooted teeth with a periodontal diseases including furcation involvement had increasing of periodontal breakdown and not responding favourably to periodontal treatment other than multi-rooted teeth without furcation or teeth with single-root, according to research. [1–3] Surgical debridement of furcation defects could result in modest clinical improvement. [4]

Recently, autologous platelet concentrates have increased popularity as a treatment for a variety of intraoral clinical problems, including periodontal defects. The main generations of APCs are platelet-rich plasma (PRP), plasma rich in growth factors (PRGF), platelet-rich fibrin (PRF) and its modifications and concentrated growth factor (CGF). [5]

The second generation or PRF has been widely used in treatment of intra bony defects, furcation defects and in root coverage procedures. [6, 7] The biologic action of platelet-rich fibrin (PRF) is an interplay between the dense fibrin matrix, high concentration of platelets and leukocytes and continuous release of growth factors and biologic mediators for more than 10 days.[8] There have been modifications to the protocol of preparation of PRF. These modifications are depends on low-speed centrifugation concept (LSCC). [9]

MATERIALS AND METHODS

Study design and registration

This study was aligned with a double-blinded, parallel arms, randomized controlled clinical trial, with 1:1 allocation ratio to Compare clinical outcomes after treatment of class II furaction defects with Advanced -PRF (A-PRF) and nano-crystalline hydroxyapatite (n-HA) (test group) versus open flap debridement (OFD) (control group). The protocol of this study was accepted by the Ethics Committee of Scientific Research, Faculty of Dentistry, Cairo University between March 2019, and March 2020 with an accepted number's (19-2-4). Screening of participants was continued till the target sample was accomplished. Identifying and Enrolment of potential subjects was done through patient's database. Accordance with the EQUATOR standards and Helsinki Declaration's ethical principles for medical research including human participants, the study was conducted and published, as revised in Fortaleza 2013

Participants:

28 furcation defects in 24 participants (11 females, 13 males, with age range 25 to 48 years) with mandibular class II furcation involvement. Patients were selected from the outpatient clinic, Department of Periodontology, Faculty of Dentistry, Cairo University. The detailed surgical technique and follow-up periods were precisely defined to all patients. Informed consent was acquired from all patients enrolled in the study who fully approved to be involved in this work and the study was reported according to CONSORT guidelines [10].

Participants who fulfilled the inclusion criteria and provided informed consent were Assigned to treatment groups by means of simple randomization with a 1:1 allocation ratio by the main supervisor. Exclusion crieteria (Patient-related criteria): pregnant or nursing women, smokers, uncooperative patients, history of periodontal treatment or systemic antibiotics within the previous 6 months, (Teeth related criteria): teeth with furcation involvement grade I, III, VI, teeth with grade III mobility. The two groups were equally prepared for both surgical procedures. Then the decision of which group would receive (A- PRF + n-HA) and which would receive OFD only was choosen in order to the randomized numbers placed in opaque sealed envelopes.

Sample size

The minimal clinically important difference in relative vertical gain in CAL between the 2 groups as estimated by the expert is 1.4. Using power of

80% and 5% significance level we will need to study 11 in each group. This number is to be increased to a sample size of 14 per group to compensate for possible losses during the follow up. Sample size calculation was achieved using PS program (Pradeep *et al*., 2009)**.**

Randomization:

Furcation defects were randomly selected to receive (A- PRF + n-HA) with OFD or OFD alone using randomization list with 1:1 allocation ratio. Using www.random.org, - (NCT03804086). A single investigator MH generated the sequences and concealed them. Sequentially numbered, similar, and opaque tightly sealed envelopes were used to hide allocation. MH was responsible for assigning the allocation of participants into the corresponding study group. All participants were enrolled and equally prepared for the surgical procedure by a single investigator (HA), the allocation was revealed (MH) to the operator (HA) according to the sequence.

Blinding:

This trial is a single-blinded.

Clinical parameters:

Clinical periodontal parameters, which were relative vertical clinical attachment loss (RVCAL) (primary outcome) and gingival recession (GR), periodontal pocket depth (PPD) were recorded using Williams graduated periodontal probe at the site of middle buccal or lingual defect and were rounded up to the nearest millimetres and relative horizontal clinical attachment loss (RHCAL), were recorded using Nabers probe measurements were reported at base line and at 3, 6 & 9 months (secondary outcome).

Relative horizonta clinical attachment level (RHCAL), relative vertical clinical attachment level (RVCAL) and gingival marginal level (GML) will be conducted from the apical level of customized acrylic stents with grooves to ensure a repeatable placement of a periodontal probe.

Postoperative pain will be measured using the Visual Analogue Scale (VAS) that measures the intensity of pain. The VAS contains a two end points including 0 ('no pain') and 10 ('pain as bad as it could possibly be'). Ask the patient to rate their pain by placing a mark to rate their current level of pain.

Treatment protocol:

Control groub

Both groubs (control and intervention), after Local anesthesia, a full thickness mucoperiosteal flap was raised by Buser periosteal elevator after intrasulcular incisions in the diseased tooth and one adjacent tooth mesial and distal using # 15c blades. Then by using mini-five and after-five Gracy curettes depridement was performed. (**Figures,1, b**).

Then the flap is repositioned and secured with the interupted and mattres sutures using 5-0 monofilament polypropylene suture material. **(figure 1, c) (figure 2, h)**

Intervention groub

After a local anethesia a full thickness mucoperiosteal flap was raised by Buser periosteal elevator after intrasulcular incisions in the diseased tooth and one adjacent tooth mesial and distal using #15c blades. Then by using mini-five and after-five Gracy curettes depridement was performed. (**Figures,1, b**).

A-PRF was prepared following the protocol, just before surgery, intravenous blood (by venipuncture of the antecubital vein) was collected in an10-mL sterile tubes without anticoagulant and you immediately centrifuged at 1300 revolutions per minute (200 x g) for 14 minutes at room temperature. Blood centrifugation immediately after collection permits the formation of a structured fibrin clot in the middle of the tube, just between the

red corpuscles at the bottom and acellular plasma (platelet-poor plasma) at the top. **(Figures,2, f)** [11]

A-PRF was simply separated from the base of red corpuscles (preserving a small red blood cell layer) by using sterile tweezers and scissors onto a sterile compress.

The resulting A-PRF clot was pressed between two pieces of sterile gauze then, the consequent membrane was placed over after filling the furcation defect with nano- crystalline hydroxyapatite bone graft**. (Figures,2, g)**

Finally, the flap is repositioned and secured with the interupted and mattres sutures using 5-0 monofilament polypropylene suture material. **(figure,1, c) (figure,2, h)**

Statistical Analysis:

Data were presented as mean, standard deviation (SD) and 95% confidence interval. Data was explored for normality using Shapiro-Wilk test. All data showed a non-normal distribution. Mann Whitney test was used to compare between control and intervention groups. Friedmann test was used for comparison between tested periods within each group. Multiple comparisons with Dunn Bonferroni were performed if Friedmann test was significant. The significance level was set at $p < 0.05$. Statistical analysis was performed with IBM® SPSS® (SPSS Inc., IBM Corporation, Armonk, NY, USA) Statistics Version 26 for Windows.

RESULTS

In both groups significant PPD reduction, V-CAL gain and H-CAL gain occurred at 6 and 9 months compared to baseline values. The mean reduction in PPD was more statistically significant at 3 and 6 months in test group compared to control group. The mean gain in V-CAL was more significant in test group compared to control group at 3, 6 and 9 months with no significant difference between groups with respect to mean gain in H-CAL. The control group showed more significant increase in gingival recession compared to test group at 6 and 9 months. At last, there is a significant difference between groups in mean postoperative pain at day 2,3,4 in favour of the test group. **(Table (1), Table (2).**

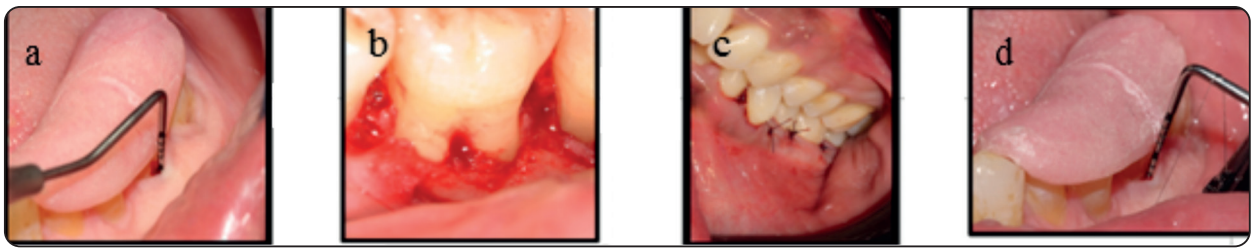

Fig. 1: (a) Clinical photograph of first molar with PPD 4 mm and CAL 5mm. (b) Clinical photograph of lower first molar after flap reflection. (c) Clinical photograph showing suturing after flap repositioning. (d) clinical photograph showing 9 months follow up of buccal furcation with 2mm PPD and 3mm VCAL (lower 6).

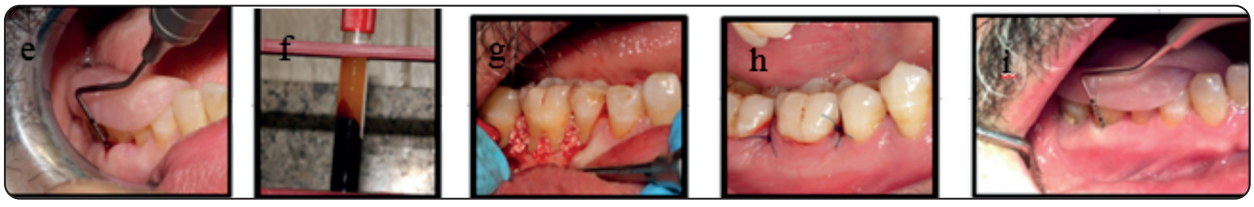

Fig. 2: (e) clinical photograph of buccal furcation with PPD 4 mm and CAL 5 mm at lower first molar (f) clinical photograph showing the tube after centrifuge the blood sample (g) placement of n-HA bone grafting material in furcation defect (h) clinical photographs showing an interrupted suture in the defect area. (i) clinical photograph of buccal furcation with PPD 2 mm and VCAL 3 mm at lower first molar after 9 months.

TABLE (1) Mean, clinical interval [95% CI] for probing pocket depth (PPD), vertical clinical attachment level (VCAL), horizontal clinical attachment level (HCAL), gingival recession (GR) (significant differences are marked with asterisk; *p < 0.05, CI: confidence interval)

		(OFD) (n = 14)		(A-PRF+n-HA) (n = 14)		P-value
		Mean (±SD)	**[95%CI]**	**Mean (±SD)**	**[95%CI]**	
PPD (mm)	Baseline	6.3^{a} (±1.8)	[5.0, 7.6]	6.6^{a} (±1.4)	[5.6, 7.5]	0.684 NS
	3 Months	6.0^{a} (±1.4)	[5.0, 6.9]	5.3^{ab} (±1.0)	[4.6, 5.9]	0.315 NS
	6 Months	5.0^{ab} (±1.1)	[4.2, 5.7]	4.1^{bc} (±0.7)	[3.6, 4.5]	0.075 NS
	9 Months	4.3^{b} (±0.9)	[3.6, 4.9]	3.7^{c} (±0.7)	[3.1, 4.2]	0.218 NS
	p-value	<0.001*		<0.001*		
changes in PPD	3 Months	-0.4^{a} (±0.8)	[-0.9, 0.2]	-1.3^{a} (±0.7)	[-1.8, -0.8]	0.007*
	6 Months	-1.4^{ab} (±1.2)	[-2.2, -0.5]	-2.5^{b} (±0.8)	[-3.1, -1.9]	0.035*
	9 Months	-2.1^{b} (±1.7)	[-3.2, -0.9]	-2.9^{b} (±1.1)	[-3.7, -2.1]	0.393 NS
	p-value	0.011*		0.001*		
V-CAL (mm)	Baseline	5.9^{a} (±1.4)	[4.9, 6.9]	6.7^{a} (±1.2)	[5.8, 7.5]	0.190 NS
	3 Months	5.8^{a} (±1.8)	[4.5, 7.1]	5.7^{ab} (±1.2)	[4.8, 6.5]	0.971 NS
	6 Months	5.6^{ab} (±2.1)	[4.1, 7.0]	4.5^{bc} (±0.8)	[3.9, 5.0]	0.393 NS
	9 Months	4.9^{b} (±1.6)	[3.7, 6.1]	4.1^{c} (±0.9)	[3.4, 4.7]	0.353 NS
	p-value	0.008*		<0.001*		
changes in VCAL	3 Months	-0.1^{a} (±0.7)	[-0.6, 0.4]	-1.0^{a} (±0.6)	[-1.4, -0.6]	0.011*
	6 Months	-0.4^{a} (±0.9)	[-1.0, 0.3]	-2.2^{b} (±0.8)	[-2.7, -1.7]	<0.001*
	9 Months	-1.0^{a} (±0.8)	[-1.6, -0.4]	-2.6^{b} (±0.8)	[-3.2, -2.0]	<0.001*
	p-value		0.078 NS	<0.001*		
H-CAL (mm)	Baseline	5.3^{a} (±1.3)	[4.4, 6.2]	5.2^{a} (±1.4)	[4.2, 6.2]	0.912 NS
	3 Months	5.2^{ab} (±1.2)	[4.3, 6.1]	4.6^{ab} (±1.0)	[3.9, 5.3]	0.247 NS
	6 Months	4.0^{bc} (±1.1)	[3.2, 4.8]	3.3^{bc} (±0.9)	[2.6, 4.0]	0.143 NS
	9 Months	3.3^{c} (±0.8)	[2.7, 3.9]	2.9^{c} (±1.1)	[2.1, 3.6]	0.393 NS
	p-value	<0.001*		<0.001*		
changes in HCAL	3 Months	-0.1^{a} (±0.3)	[-0.3, 0.1]	-0.6^{a} (±0.7)	[-1.1, -0.1]	
	6 Months	-1.3^{b} (±0.7)	[-1.8, -0.8	-1.9^{b} (±0.7)	[-2.4, -1.4]	
	9 Months	-2.0^{b} (±0.8)	[-2.6, -1.4]	-2.4^{b} (±0.6)	[-2.8, -1.9]	
	p-value	<0.001*			<0.001*	
GR (mm)	Baseline	0.8^{a} (±0.4)	[0.5, 1.1]	-0.1^{a} (±1.1)	[-0.9, 0.7]	0.043*
	3 Months	-0.2^{ab} (±0.6)	[-0.7, 0.3]	-0.1^{a} (1.0±)	[-0.8, 0.6]	0.796 NS
	6 Months	-1.0^{b} (±0.8)	[-1.6, -0.4]	-0.4^{a} (0.5±)	[-0.8, 0.0]	0.123 NS
	9 Months	-1.1^{b} (±0.7)	[-1.6, -0.6]	-0.4^{a} (0.5±)	[-0.8, 0.0]	0.052 NS

Changes in GR					
3 Months	-1.0[a] (±0.7)	[-1.5, -0.5]	0.0[a] (±1.5)	[-1.1, 1.1]	0.063 NS
6 Months	-1.8[b] (0.9±)	[-2.5, -1.1]	-0.3[a] (±0.9)	[-1.0, 0.4]	0.004*
9 Months	-1.9[b] (±0.9)	[-2.5, -1.3]	-0.3[a] (±0.9)	[-1.0, 0.4]	0.002*
p-value	0.031*		1.00 NS		

Table 2: Mean, standard deviation and 95% CI of difference in pain for different tested groups.

	(OFD) (n = 14)		**(A-PRF+n-HA) (n = 14)**		**P-value**
	Mean (±SD)	**[95%CI]**	**Mean (±SD)**	**[95%CI]**	
day 0	6.2[a] (±1.5)	[4.6, 7.7]	4.4[a] (±1.5)	[2.5, 6.3]	0.126 NS
day 1	5.7[a] (±1.4)	[4.2, 7.1]	4.0[ab] (±1.6)	[2.0, 6.0]	0.126 NS
day 2	4.5[ab] (±0.5)	[3.9, 5.1]	2.6[ab] (±1.1)	[1.2, 4.0]	0.009*
day 3	4.0[abc] (±0.6)	[3.3, 4.7]	2.2[ab] (±1.1)	[0.8, 3.6]	0.030*
day 4	2.7[abc] (±1.0)	[1.6, 3.8]	1.0[ab] (±0.7)	[0.1, 1.9]	0.030*
day 5	2.0[bc] (±0.6)	[1.3, 2.7]	1.0[ab] (±0.7)	[0.1, 1.9]	0.052 NS
day 6	1.3[c] (±1.2)	[0.1, 2.6]	0.8[bc] (±0.8)	[-0.2, 1.8]	0.537 NS
day 7	1.0[c] (±0.9)	[0.1, 1.9]	0.2[c] (±0.4)	[-0.4, 0.8]	0.177 NS
p-Value	<0.001*		<0.001*		

DISCUSSION

Advanced platelet rich fibrin (A-PRF) performs the latest generation of platelet concentrates that provide concentrated autologous growth factors in a fibrin mesh entrapping platelets and neutrophils. The preparation of A-PRF is based upon low-speed centrifugation concept (LSCC). The reduction in the relative centrifugation force would promote a more uniform distribution of platelets in the fibrin matrix in contrast to high centrifugation forces that tend to push the platelets to sediment close to the RBC fraction. [8] PRF and A-PRF provide a local delivery of different isoforms of PDGF (PDGF-AA, PDGF-AB, PDGF-BB), TGF-β1, TGF-β2, EGF, IGF, VGF and FGF-2. [8], [11], [12]

In comparison to PRF the second regeneration and the widely used platelet concentrate in periodontal regeneration the A-PRF provides higher total quantity and more sustained release of growth factors such as PGF-AA, PDGF- AB, TGF -β, VGF and EGF. [13] So**,** the present study A-PRF was applied in combination with nano hydroxyapatite bone graft. The decision to include the bone graft in the intervention group was based upon the systematic review that showed that mandibular class II furcation defects treated with combination of GTR with bone graft demonstrated more favorable gain in VCAL and horizontal bone gain compared to GTR alone. [14]

The choice of n-HA bone graft was driven by its favourable biological properties being biocompatible and osteoconductive. Its nano-sized dimensions promote higher surface to volume ratio that can attract and bind osteoblasts and PDL cells and promote graft resorption and replacement by new bone when implanted in bone defects. [15]

The management of furcation defects with surgical debridement through OFD enhanced clinical outcomes producing significant mean reduction in PPD 2.1±1.7mm, mean gain in VCAL1.0 ±0.8mm

and mean gain in HCAL 2.0 ±0.8 mm 9 months after treatment of class II furcation involvement compared to baseline values. Changes coincide with mean PPD reduction 2.88 ± 0.67mm, mean gain in VCAL 1.27 ± 0.46mm and mean gain in HCAL 1.88 ± 0.75mm. reported by Sharma and Pradeep [16] Similar findings were also reported by Bajaj. [17] in a systematic review which measured the clinical response of class II mandibular furcation defects to OFD in randomized controlled clinical trials. [4]

In the test group the application of A-PRF membrane in combination with n-HA bone graft produced significant clinical improvement 9 months after treatment of class II furcation involvement compared to the baseline. There was significant mean reduction in mean PPD 2.9 ±1.1mm with significant mean gain in VCAL 2.6 ± 0.8mm and significant mean gain in HCAL 2.4 ±0.6mm. These findings are in line with the study conducted **by Lohi** This study applied a composite of bioactive glass and hydroxyapatite bone graft and PRF membrane to mandibular class II furcation defects. [18] After 6 months mean PPD reduction was 3.37 ± 1.06mm while mean gain in VACL was 3.00±0.92mm. The findings in the present study coincide with the findings of **Rani** after treatment of class II furcation defects with B tricalcium phosphate bone graft and PRF membrane. The reported mean PPD reduction reached 2.80 ± 1.93 mm and mean gain in VCAL reached 3.00 ± 1.44mm after 9 months. [19]

In the present study the mean HCAL gain was 2.4 ±0.6mm 9 months in the test group after treatment. This agrees with studies that evaluated the combination of bone grafts and autologous platelet concentrates. In a study by **Qiao** the application of concentrated growth factor and xenograft induced 2.10±1.89 mean gain in HCAL and 2.78±1.66 mm mean gain in VCAL one year after treatment of class II . [20] The study by **Mansouri** evaluated the application of plasma rich in growth factors and bovine porous bone mineral and reported 1.65±1.24mm mean gain in VCAL and 1.29±1.30mm mean gain in HCAL [21]. The present study application of A-PRF as a membrane in combination with n-HA bone graft produced more significant mean gain in VCAL, and reduction in mean PPD compared to OFD. This coincide with systematic reviews that demonstrated improved clinical outcomes when PRF was applied compared to OFD alone. [6, 7]

In the present study there is no, significant difference was detected with respect to changes in HCAL in the test group compared with the control group. Similar changes were reported by **Agarwal** That there is no difference with respect to changes in HCAL between the furcation defects treated with OFD and combination of DFDBA and PRF. [22] In contrast, a significant difference in mean HCAL gain between class II mandibular furcation defects treated with OFD alone compared to OFD and combination of PRF and Hydroxyapatite bone graft in favors of the PRF group [22]. A possible explanation for the findings in the present study would be formation and maturation of supracrestal tissue that would interfere with penetration of the Naber's probe [23].

The present study showed the stability of the gingival margin with minimal change in mean gingival recession in the test group (0.3 ±0.9 mm) compared to the control that demonstrated significant recession (1.9 ±0.9 mm) 9 months after treatment of class II furcation involvemet. Similar findings were recorded following application of PRF compared to OFD in treatment of class II mandibular furcation defects [16]**.** This could be related to the adhesive function of the fibrin matrix providing mechanical support for the flap margin maintaining its position with minimal tissue shrinkage during healing as postulated by Del Corso [24]**.**

The test group reported less mean postoperative pain at day 2,3,4 after surgical intervention compared to control group. It is founded that serotonin

released from activated platelets maybe responsible for decreased pain, as mentioned by Everts [25]. Exogenous application of Platelet-leukocyte gel during open subacromial decompression contributes to enhanced patient outcome. A prospective randomized double-blinded study [25].

The limitations of the present study include the absence of a group treated with A-PRF alone as the application of PRF alone has demonstrated effectiveness in treatment of furcation class II defects [7]. Another limitation is the exclusion of maxillary class II furcation defects. The inclusion of maxillary defects as a separate group would have substantiated the possible effectiveness of the recent generation of platelet concentrates in management of the challenging furcation defects.

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