

Evaluation of Leukocyte Platelet Rich Fibrin with Antimicrobial Photodynamic Therapy in the Treatment of Furcation Involvement: A Randomised Clinical Study

Walid Elamrousy^{1*}, Ahmed Mortada²

¹Periodontology Department, Faculty of Dentistry, Kafrelsheikh University, Kafrelsheikh, Egypt

² Periodontology Department, Faculty of Dentistry, Assiut University, Assiut, Egypt

*waled_hammed@den.kfs.edu.eg

ABSTRACT

Objectives: The aim of the present study is to assess the clinical effectiveness of Leukocyte-Platelet Rich Fibrin (L-PRF) combined with antimicrobial Photo-Dynamic Therapy (aPDT) compared with the sole use of L-PRF in treatment of furcation grade II involvement.

Materials and methods: Twenty-four patients with Grade II furcation involvement of the first or second mandibular molars were randomly assigned immediately before surgery into two groups (12 patients each). Control group received L-PRF alone as a sole graft material, while the study group used L-PRF in combination with aPDT for the treatment of the periodontally involved furcation defects.

Results: The results of the present study demonstrated a significant improvement of clinical and radiographic parameters at 6 months for both groups when compared to baseline measurements. Intergroup comparison revealed a statistical significant gain of the radiographic bone defects of the study group when compared to the control group

Conclusion: This study concluded that the use of L-PRF only with open flap debridement is considered an effective method in treatment of grade II furcation involvement. Also the aPDT combined with LPRF and open flap debridement showed a promising clinical and radiographic results in cases of furcation grade II involvement.

Keywords

Furcation involvement; leukocyte platelet rich fibrin; antimicrobial photodynamic therapy; regeneration.

Introduction

Inter-radicular loss of attachment and bone resorption resulting from plaque induced periodontal disease is named Furcation involvement[1]. Treatment of the periodontally involved furcation represents a challenge for clinicians due to anatomy and morphology complexity[2]. Grade II furcation treatment using different regenerative techniques successfully improved bone fill and attachment gain[3].

The use of autologous platelet concentrates as a source of high concentrations of growth factors improved soft and hard tissue healing through promoting bone fill and attachment gain[4]. Plasma rich in growth factors (PRGF), platelet rich plasma (PRP), concentrated growth factors (CGF) and platelet rich fibrin (PRF) are the most common forms of autologous platelets concentrates[5].

Preparation of PRP and PRGF is time-consuming, technically complicated and requires bovine thrombin or CaCl_2 which inhibits early wound healing. Furthermore, failure of homogenous leukocytes incorporation and weak fibrin network leading to limited bone regeneration potential[6].

Preparation of PRF is simple; easy to produce; no need for anticoagulants or bovine thrombin and no biochemical manipulation of blood is required[7]. PRF is characterized by sustained release of relative amount of various cytokines when compared to PRP and PRGF[6].

L-PRF is considered the new generation of autologous platelet concentrate, as it consists of fibrin mesh containing leukocytes, growth factors, cytokines and proteins[8]. L-PRF has a prolonged release of key growth factors ranging from 1 week to 28 days, thus stimulates wound healing and promotes tissue regeneration[9,10]. Also L-PRF stimulates *in vitro* differentiation and proliferation of osteoblasts[11].

The use of antimicrobial photodynamic therapy (aPDT) could be a promising therapeutic approach in periodontal therapy because of photosensitizers high affinity to bacterial membranes[12]. Moreover, aPDT demonstrated short half-life, short reactive oxygen species diffusion paths and rendered the unlikely bacterial resistance [13].

aPDT is effective for killing resistant bacteria in complex subgingival plaque biofilms by damaging the microorganisms membrane and DNA through releasing of singlet oxygen from oxygen-releasing dyes[14]. The main applications of aPDT in dentistry include root canals disinfection, treatment of periodontal pockets and peri-implantitis [15].

No controlled trials have been performed to prove the benefits of a combination of aPDT and L-PRF on the treatment of periodontal diseases. To the best of our knowledge, the present research is the first study that will evaluate the combined effect of aPDT and L-PRF on the treatment of furcation intrabony defects.

MATERIALS AND METHODS

Study design and eligibility criteria

The present study was designed as a randomised, double-blinded study (patient and data collection examiner). Twenty-four participants from the Periodontology Department, Kafrelsheikh University, Egypt and suffering from a moderate-to-severe chronic periodontitis with furcation involvement (Grade II) of the first or the second mandibular molars aged from 18 to 50 years and had 3 mm at least vertical probing depth were recruited in this study.

Exclusion criteria's were: using medications that interfere with wound healing, presence of systemic condition, clotting and bleeding disorders, smokers, the presence of anatomical complexity that interfere with proper scaling and root planning in the selected teeth, the presence of caries or restorations in furcal area, Grade II mobility or more, any contraindication for periodontal surgery, allergy to any used materials in current study, the presence of radiographic periapical lesions and the unwillingness of the patient to accept a periodontal surgery.

Randomization and blindness

Patients were randomly assigned into two groups (12 patients each) using computer-assessed randomization software (Random Allocation Version 1.0). Control group used L-PRF alone, and the study group applied L-PRF combined with aPDT. One clinician performed all surgical interventions. Another blinded clinician measured the periodontal indices. Dental Kafrelsheikh University research ethical committee accepted the study (KD/09/20). Registration in the clinical trial registry was done (RCT: NCT04842188).

Study protocol

All enrolled participants approached a written consent. All participants received presurgical scaling with root planing and oral hygiene maintenance instructions [figure 1]. If needed, occlusal correction and adjustment was performed. All the surgical procedures had been performed by one periodontist as the following: intrasulcular incision followed by mucoperiosteal flap elevation [figure 2], then granulation tissue debridement followed by subgingival scaling and subgingival root planing. After that, the patient received the arms (either L-PRF alone in the control group [figure 3] or L-PRF with aPDT in the study group) based on the predefined random allocation. Finally, suturing of the coronally positioned flap with silk 3-0 [figure 4], and the patients were prescribed postoperative antiseptic mouthwash and antibiotic.

L-PRF preparation

Blood Samples were placed in 9 mL plastic tubes coated with glass (Becton Dickinson Vacutainer, NJ, USA). The blood was instantly centrifuged at 2700rpm using a table centrifuge for 12 minutes (DLAB DM0506 low-speed centrifuge, DLAB Scientific Co., Ltd., China). The L-PRF clot was taken from the tube middle portion and any red blood cells remnants were scraped off using sterile gauze.

aPDT: [figure 5]

Low-level laser red light therapy was used in the current research (Photo Activated Disinfection Light F3WW, KSD Medical Instrument Co., Ltd. Guangdong, China) with toluidine blue O.

Primary and secondary end points

Clinical assessments:

Vertical pocket depth (VPD), vertical clinical attachment level (VCAL), gingival recession (REC) and furcation horizontal component (FHC) were measured clinically at baseline and after 6months postoperatively.

Radiologic evaluation:

Bone defect height, depth, width and volume were recorded radiographically using CBCT at baseline and after 6 months postoperatively.

Sample size and statistical analysis

Using an Altman's nomogram, we estimated that 12 patients should be enrolled to achieve 80% power with standardized difference of 1.4 between the two research arms. Paired *t*-test was used to assess two-time parameter changes and to compare mean differences between 2 different intervals of the one group. Changes between the two different groups were compared by independent sample *t*-test. IBM SPSS software version 20 (IBM Corp., Armonk, NY, USA) has been utilized for statistical analysis. $P < 0.001$ was considered statistically significant.



Fig.1: preoperative view, Fig.2: flap reflection, Fig.3: Application of L-PRF, Fig.4: suturing



Fig.5: Photo Activated Disinfection Light F3WW

Results:

Twelve patients were finally recruited for each group in this present study from April 2020 till April 2021. Overall, clinical and radiographic parameters for both groups were significantly improved except the REC that was insignificantly deteriorated. Intergroup comparison revealed insignificant changes in the clinical parameters, whereas radiographic measurements revealed significant improvements of the study group when compared to the control group [tables 1-4].

In this clinical trial, a total number of twenty-four patients were finally enrolled. Eleven patients were female and thirteen patients were male their age ranged from 26 to 50 years with 34 years mean age [tables 1].

The mean VCAL at baseline was 5.25 ± 1.05 mm and 5.58 ± 0.90 mm for the control and the study groups respectively; after 6 months postoperatively VCAL values significantly dropped to 3.08 ± 0.66 mm and 2.50 ± 0.67 mm for the control and the study groups respectively ($P < .001$) [table 2].

Comparing the mean VPD from baseline to 6 months yielded statistically significant reductions in VPD from 4.67 ± 0.77 mm to 2.67 ± 0.65 mm for the control group, and from 5.25 ± 1.13 mm to 2.08 ± 0.28 mm for the study group ($P < .001$) [table 2].

The baseline measurements of FHC for the control and study groups were 4.17 ± 0.71 mm, 4.25 ± 0.62 mm respectively,

while the 6 months measurements were significantly decreased to 2.50 ± 0.67 mm, 1.83 ± 0.57 mm respectively ($P < .001$) [table 2, graph 1].

Comparisons of the mean REC values showed non-significant increase from 0.12 ± 0.22 mm at baseline to 0.58 ± 0.41 mm after 6 months for the control group. Also, the study group showed non-significant increase of the mean recession values from 0.16 ± 0.24 mm at baseline to 0.54 ± 0.39 mm after 6 months [table 2].

At 6 months for control and study groups, the mean VCAL gain was 2.17 ± 0.71 mm and 3.08 ± 0.79 mm, respectively, and the mean VPD reduction was 2.00 ± 0.85 mm and 3.17 ± 1.03 mm, respectively. Also at 6 months for control and study groups, the mean decrease in FHC was 1.67 ± 0.49 mm and 2.42 ± 0.51 mm, respectively, and the mean REC increase was 0.45 ± 0.33 mm and 0.37 ± 0.43 mm, respectively. The mean reductions in VPD and FHC at 6 months were not statistically different between the study and control groups. Additionally, VCAL gain and increase of REC values were not significantly different at 6 months when comparing the control and study groups [Table 3].

The radiographic bone defect height at baseline was 2.95 ± 0.04 mm for control group and 2.96 ± 0.04 mm for study group. At 6 months, it was statistically reduced to 2.02 ± 0.02 mm for control group and 1.92 ± 0.02 mm for study group [Table 4].

Regarding the radiographic bone defect depth, the results were significantly dropped from 2.85 ± 0.03 mm at baseline to 1.97 ± 0.03 mm at 6 months for control group, and from 2.87 ± 0.02 mm at baseline to 1.85 ± 0.02 mm at 6 months for study group [Table 4].

The baseline measurements of radiographic bone defect width for the control and study groups were 2.24 ± 0.02 mm, 2.34 ± 0.20 mm respectively, while the 6 months measurements were significantly decreased to 1.73 ± 0.01 mm, 1.52 ± 0.01 mm respectively ($P < .001$) [Table 4].

The radiographic readings of the bone defect volume demonstrated significant reduction from 16.59 ± 1.66 mm³, 17.67 ± 1.40 mm³ at baseline for control and study groups respectively to 5.28 ± 0.38 mm³, 7.39 ± 0.45 mm³ at 6 months for control and study groups respectively [Table 4].

Upon comparing 6 months means of radiographic changes with baseline means, there was a statistically significant decrease in all radiographic parameters (bone defect height, depth, width and volume) in both groups ($P < .001$) [Table 5].

At 6 months for control and study groups, radiographically the mean bone defect height fill was 0.92 ± 0.02 mm and 1.03 ± 0.02 mm, respectively, and the mean radiographic bone defect depth reduction was 0.87 ± 0.24 mm and 1.02 ± 0.27 mm, respectively. Moreover, the mean gain in radiographic bone defect width was 0.50 ± 0.03 mm and 0.81 ± 0.02 mm, respectively, and the mean radiographic bone defect volume fill was 9.19 ± 1.72 mm³ and 12.38 ± 1.60 mm³, respectively [Table 5].

Upon comparing the mean radiographic reductions in bone defect height, depth, width and volume at 6 months, the study group demonstrated significant bone fill when compared to the control group ($p < 0.001$) [Table 5].

Table 1: Basic demographic characteristics of the study population

Parameter	Control Group	Study Group
Number	12	12
Mean age $\pm$ SD (years)	34.5 ± 9.05	33.75 ± 6.90
Range (years)	27-50	26-48
Males/Females	6/6	5/7

SD: Standard Deviation

Table 2: Mean $\pm$ SD values of clinical parameters of both groups at baseline and 6 months postoperatively

Parameter	Control Group Mean $\pm$ SD	P	Study Group Mean $\pm$ SD	P
VCAL				
Baseline	5.25 ± 1.05	$<0.001^*$	5.58 ± 0.90	$<0.001^*$
6 months	3.08 ± 0.66		2.50 ± 0.67	
VPD				
Baseline	4.67 ± 0.77	$<0.001^*$	5.25 ± 1.13	$<0.001^*$
6 months	2.67 ± 0.65		2.08 ± 0.28	
FHC				
Baseline	4.17 ± 0.71	$<0.001^*$	4.25 ± 0.62	$<0.001^*$
6 months	2.50 ± 0.67		1.83 ± 0.57	
REC				
Baseline	0.12 ± 0.22	$=0.001$	0.16 ± 0.24	>0.001
6 months	0.58 ± 0.41	(NS)	0.54 ± 0.39	(NS)

Table 3: Mean changes in the clinical parameters over 6-month period among the groups

Parameter	Control Group Mean±SD	Study Group Mean±SD	P
VCAL gain	2.17±0.71	3.08±0.79	>0.001 (NS)
VPD Reduction	2.00±0.85	3.17±1.03	>0.001 (NS)
FHC Reduction	1.67±0.49	2.42±0.51	=0.001(NS)
REC increase	0.45±0.33	0.37±0.43	>0.001 (NS)

VCAL: Vertical Clinical Attachment Level, VPD: Vertical Pocket Depth, FHC: Furcation Horizontal Component, REC: Gingival Recession, SD: Standard Deviation, *: Significant difference, NS: No significant difference

Table 4: Mean±SD values of radiographic parameters of both groups at baseline and 6 months postoperatively

Parameter	Control Group Mean±SD	P	Study Group Mean±SD	P
Defect height				
Baseline	2.95±0.04	<0.001*	2.96±0.04	<0.001*
6 months	2.02±0.02		1.92±0.02	
Defect depth				
Baseline	2.85±0.03	<0.001*	2.87±0.02	<0.001*
6 months	1.97±0.03		1.85±0.02	
Defect width				
Baseline	2.24±0.02	<0.001*	2.34±0.20	<0.001*
6 months	1.73±0.01		1.52±0.01	
Defect volume				
Baseline	16.59±1.66	<0.001*	17.67±1.40	<0.001*
6 months	7.39±0.45		5.28±0.38	

SD: Standard Deviation, *: Statistical significant difference

NS: Not statistically significantly different

Table 5: Mean changes in the radiographic parameters over 6-month period among the Groups

Parameter	Control Group Mean±SD	Study Group Mean±SD	P
Bone defect height fill	0.92±0.02	1.03±0.02	<0.001*
Bone defect depth fill	0.87±0.24	1.02±0.27	<0.001*
Bone defect width fill	0.50±0.03	0.81±0.20	<0.001*
Bone defect volume fill	9.19±1.72	12.38±1.60	<0.001*

SD: Standard Deviation, *: Statistical significant difference

NS: Not statistically significantly different

DISCUSSION

The current study was conducted on mandibular first or second molars with grade II involvement of the furcation. The research arms consisted of either L-PRF with aPDT or L-PRf interventions.

The outcomes of the periodontal parameters of VCAL, VPD, FHC, REC and CBCT volumetric analysis of the bone defects have helped us to derive significant conclusions. The baseline-investigated parameters demonstrated no substantial differences between L-PRF group and L-PRF+aPDT group indicating effective randomization.

All the participants in this present study demonstrated clinical healthy gingiva and good oral hygiene throughout the study duration. This could be attributed to the continuous patient motivation and reinforcement to maintain excellent oral hygiene.

In a clinical study of Raja et al. [16], PRF was found to be advantageous over PRP because of ease of preparation, cost effective, and no need to biochemical handling of blood and cytotoxic characteristics. Biswas et al. [17], compared PRF and Nova bone in cases with class II furcation defects, the achieved VCAL gain after 6 months was 3.7 ± 0.11 mm and 2.1 ± 0.12 mm respectively indicating that PRF had better regenerative characteristics than the synthetic alloplasts.

In the present study, a significant gain of VCAL and significant reduction of VPD was observed in both research groups after 6 months. Upon comparing the two groups at 6 months no statistical significant differences of VCAL gain and VPD reduction. Although the results of our study enabled the patient to maintain good oral hygiene and perform better plaque control, this gain in VCAL and the reduction of VPD were lesser to that noticed in Sharma et al. [18] study that revealed 4.056 ± 0.416 mm drop in PPD and 2.333 ± 0.485 mm gain in RVCAL after 9 months of application of PRF in grade II furcation defects. Similarly, in a study by Agarwal et al. [19], changes in VCAL and VPD after 9 months were found to be 3.55 ± 1.05 mm and 3.80 ± 0.77 mm respectively, which was higher than results obtained from our present study. The lesser observation in our study could be attributed to the shorter time

span for evaluation (6 months). The results reported by Wanikar and colleagues [20] were close to that obtained from the control group but much lesser than the study group in our present study as Wanikar et al. noticed 1.09 ± 0.64 mm gain in RVCAL and 1.85 ± 0.59 mm reduction of VPD after 6 months of application of PRF for the treatment of class II furcation involvement. The results recorded in our present study were higher to that obtained by Sharma & Pradeep [21], Bajaj et al. [22], both articles, compared open flap debridement to debridement with L-PRF, the results showed mean VPD reduction 1.9 mm and mean VCAL gain 1.3 mm.

At 6 months, non-significantly higher mean FHC reduction was observed for PRF+ aPDT group as compared to the PRF group. These results were similar to the results obtained by Shah et al. [23] who compared bone allograft (demineralized freeze-dried) alone or combined with chorion membrane in treating of furcation grade II defects and found significant reduction of the mean FHC by 1.65 ± 0.58 mm and 2.45 ± 0.82 mm, respectively. On the other hand, present study results were lesser than that noticed by Kanoriya et al. [24], who compared the potential of open flap with debridement alone, PRF, and PRF+ Alendronate in degree II mandibular molars furcation defects and revealed a significant improvement in clinical parameters at 9 month in both test groups. The authors reported VPD and FHC reduction of 4.4 ± 0.57 mm and 4.12 ± 0.6 mm respectively, which was the highest with the combined therapy (PRF + Alendronate+ open flap debridement) signifying the accessory efficiency of integrated different approaches serving growth mediating factors for regeneration.

A good response of furcation defect treatment associated with L-PRF in this study could be attributed to the polymerised tetramolecular structure of L-PRF incorporating platelets, cytokines and leukocytes, and circulating stem cells. In addition, The slow release of various glycoproteins and growth factors like platelet- derived growth factor-AB, vascular endothelial growth factor and transforming growth factor-1b, over 14 days was achieved from L-PRF due to organization of L-PRF into dense fibrin scaffold [25]. L-PRF was also used as a sole graft biomaterial scaffold for periodontal regeneration due to cytokines release, fibroblasts and endothelial cells attraction and activation, and bone-forming cells stimulation [26,27]. Also, L-PRF increases the level of alkaline phosphates that stimulates osteoblastic activity and induces bone formation [28]. Moreover, L-PRF stimulates neovascularization with fibroblasts proliferation and could aid in periodontal regeneration by formation of healthy granulation tissue [28].

Therefore, even though, the use of both L-PRF alone as well as L-PRF+aPDT for the treatment of furcation defects lead to statistical significant improvements in clinical parameters, a better regenerative potential of using L-PRF+aPDT could be confirmed radiographically.

To the authors knowledge no other previous studies have evaluated the amount of defect fill obtained with L-PRF or L-PRF+aPDT by using CBCT. Hence, the present study results could not be correlated with any previous research.

Pajnigara et al. reported that CBCT proves to be far superior in accurate diagnosis and evaluation of the treatment outcomes in furcation defects [29]. Walter et al. [30] revealed that 84% of the CBCT data was accurate upon comparing to intra-surgical data. Similar to previous literatures, CBCT evaluation in this study has enabled us to assess the healing properties and regeneration volumetrically without surgical invasion [31,32].

In the present study, possible reasons for better results in L-PRF+aPDT group could be attributed to the additional aPDT influence over the local tissues. It was observed in previously mentioned studies that aPDT has a supportive role in periodontal healing by direct action on bacterial apoptosis and indirectly by decrease the action of host response [33,34]. Another effect founded on bacteria was the breakdown of polysaccharides in the cytoplasmic membrane of bacteria [34], besides the killing effect on bacterial biomolecules [35]. Moreover, aPDT was noted to be responsible for the proliferation of fibroblasts, elastin and collagen, which has a positive effect on periodontal regeneration [36], which matched with the results of the study group in current study. Moreover, Fekrazad et al. [37] evaluated the single dose effect of aPDT with different wavelengths on the bone marrow mesenchymal stem cells proliferation and its differentiation into bone. The results revealed that red and infrared low level laser lights had a stimulatory function on osteogenesis.

Among the different photosensitizers in the periodontal field, toluidine blue O has been used in the present study, because of its well documented antimicrobial effect on oral pathogens [38,39]. Nielsen et al. [40], compared the effect of aPDT using toluidine blue O and Riboflavin on different periodontal pathogens. The results revealed a strong full killing power with toluidine blue O on all investigated organisms including *A. actinomycetemcomitans*, *P. intermedia* and *P. gingivalis* compared to Riboflavin.

Martins et al. [41] examined the power of aPDT by using 660nm diode laser and phenothiazine chloride dye combined with open flap debridement and resulted in clinical improvement of the condition and red complex bacteria reduction which is augmented in the present study results.

Dalvi et al. [42] evaluated the efficacy of aPDT single session adjunctive to open debridement in chronic periodontitis patients. The study results recorded a statistically significant improvement of clinical parameters. Moreover, test group showed a significant improvement in VCAL and gingival index.

Based on the search results of Gholami et al.[43], an obvious positive action of aPDT with low-level light therapies was detected on regeneration of periodontal soft and hard tissues.

Conclusion:

Within the present study limits, we concluded that using L-PRF as a sole graft material after open flap debridement is considered to be an effective method in treating furcation involvement grade II showing clinical and radiographic improvement. Also the application of aPDT combined with L-PRF and open flap debridement showed promising radiographic results in cases of furcation involvement grade II.

Further studies are required with larger sample size and for microbial assessment of the putative periodontal pathogens before and after this treatment modality.

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