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Original Article

The effect of plasma rich in growth factors (PRGF) in the treatment of periodontal three-walled intrabony defects

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Abstract

Introduction: The aim of periodontal treatment is to regenerate periodontium. Regenerative treatments include the use of plasma that is rich in growth factors (PRGF).

Materials & Methods: In a randomized clinical trial, 20 three-walled intrabony defects from five patients with moderate periodontitis were randomly assigned to three groups. Patients in the control group underwent debridement of lesions. In the first treatment group, the defects were debrided and cenomembrane was applied. The third group was treated with debridement, PRGF and cenomembrane. Measures of vertical probing depth (VPD), vertical clinical attachment level (VCAL), gingival index (GI; Sinless and Loe) and radiographic index (by digital subtraction) were made preoperatively and 6 months post-surgery. Wilcoxon signed-ranks and Chi-square tests were used for analyzing quantitative and qualitative variables, respectively.

Results: All three groups showed improvements in all measures except GI. Intra-group comparison for clinical attachment level (CAL) indicated significant difference in all groups before and after surgery ($P<0.05$); there was no correlation in CAL among groups before surgery Intra-group comparison demonstrated significant differences in all three groups before and after surgery ($P<0.001$). A statistically significant difference was found in radiographic indices among the groups post-surgery ($P=0.009$).

Conclusion: The use of PRGF was associated with improvements in all parameters but not for GI.

Keywords: Periodontitis, Periodontium , Plasma

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تاثیر استفاده از پلاسمای غنی از فاکتور رشد در درمان ضایعات استخوانی سه دیواره ی پریودنتال

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چکیده

مقدمه: هدف ایده آل درمان پریودنتال بازسازی کامل بافت می‌باشد که روش‌های رژنراتیو مثل پلاسمای غنی از فاکتور رشد (PRGF) این هدف را دنبال می کند.

مواد و روش ها: در یک مطالعه‌ی کارآزمایی بالینی تصادفی، ۲۰ ضایعه‌ی استخوانی با سه دیواره پریودنتالی از ۵ بیمار با پریودنتیت متوسط به طور تصادفی به سه گروه تقسیم شدند. در گروه کنترل دبریدمان به تنهایی، در گروه دوم دبریدمان و cenomembrane و در گروه سوم بعد از دبریدمانPRGF ، cenomembrane بکار برده شد. عمق پروبینگ پاکت، حد چسبندگی کلینیکی، اندکس لثه ای و رادیوگرافیک (با دیجیتال سابترکشن) در ابتدا و ۶ ماه بعد، اندازه‌گیری شد. برای بررسی متغیرهای کمی و کیفی به ترتیب تست ویلکاکسون و مربع کای استفاده گردید.

یافته ها: در همه‌ی گروه ها بهبود در پارامترهای ذکر شده به جز اندکس لثه‌ای دیده شد. در مقایسه درون گروهی، ارتباط معنی-داری بین حد چسبندگی کلینیکی قبل و بعد از جراحی در همه گروه‌ها دیده شد ($P<0.05$). اما بین سه گروه از لحاظ حد چسبندگی کلینیکی قبل از جراحی ارتباطی یافت نشد. ارتباط معنی داری بین سه گروه در عمق پروبینگ پاکت قبل و بعد از جراحی دیده نشد. اما در مقایسه درون گروهی ارتباط معنی‌داری قبل و بعد از جراحی در مورد عمق پاکت در سه گروه نشان داده شد ($P<0.001$). اختلاف آماری معنی‌داری در شاخص‌های رادیوگرافی بین گروه‌ها، بعد از عمل جراحی یافت شد ($P=0.009$).

نتیجه گیری: PRGF سبب بهبودی در تمامی پارامترهای اندازه گیری شده به جز اندکس لثه ای میشود.

واژگان کلیدی: پریودنتیت، پریودنشیوم، پلاسما

Introduction

Angular intrabony defects are a sign of periodontal disease progression. To date, many techniques including resective and regenerative procedures have been suggested for treating periodontal intrabony defects. Resective techniques eliminate granulation tissue, but do not regenerate periodontium. The goal of periodontal treatment is to regenerate the damaged periodontal structures.[1] Many studies indicate that the growth factors affect proliferation, chemotaxis, cell differentiation and synthesis of extracellular matrix, as well as the vital cells involved in wound healing, and have a role in both periodontal healing and regeneration.[2, 3]

Biologically active endogenous proteins offer a new approach to tissue regeneration. In 1999, Anitua[2] described a new technique for preparing plasma rich in growth factors (PRGF). This 100% autologous preparation is enriched with biological mediators that accelerate regeneration of both hard and soft tissues. Unlike platelet-rich fibrin (PRF), it contains no leukocytes or inflammatory products,[2] adhesive proteins such as fibrinogen, fibronectin and thrombospondin-1. These act as scaffold materials and attract platelets and undifferentiated cells. The platelets provide growth factors such as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β) vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF) and granulocyte-macrophage colony stimulating factor (GM-CSF).[3]

In vitro studies suggest that PRGF initiates fibroblasts proliferation[4] and promotes wound healing in epithelial tissue,[5] tendons and muscles.[6] Other studies demonstrate a positive effect of PRGF on bone maturation .[7] Thorat et al. used PRF to treat intrabony defects, and found that it has improved the periodontal regeneration [8], which is similar to that of Sharma et al. [9] Yilmaz et al. applied PRGF for mandibular intrabony defects, and observed a reduction in probing pocket depth (PPD), an increase in clinical attachment level and bone gain.[10] Del Fabbro et al. showed that using platelet-rich plasma (PRP) in periodontal lesions, in combination with graft material, has an augmented

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effect on bone healing, but not in guided-tissue regeneration (GTR) procedures.[11]

Taking into account the known effects of PRGF on hard tissue healing, this study aimed to evaluate the treatment in periodontal three-walled intrabony defects.

Materials & Methods

Case selection: This randomized clinical trial was approved by ethics committee of Babol Medical University (Grant No. 1395159 and ethics number mubabol.rec.1395.159). We selected 20 three-walled intrabony defects from 5 patients with moderate periodontitis. The procedure and rationale were explained to the patients and their informed consent was obtained.

Inclusion and exclusion criteria: Patients were selected if they had a three-walled intrabony defect on the buccal or lingual side of an upper or lower first or second premolar or molar.

Exclusion criteria were: any diagnosed systemic disorders, requirement for prophylactic antibiotics to prevent bacterial endocarditis, taking drugs that interfere with periodontal wound healing; smoking, any periodontal surgery contraindication, teeth with anatomical complications (e.g. cervical enamel projections), bifurcation ridges, accessory canal and concavity, hemi-septal bony defects, caries in the roots of teeth adjacent to intrabony defects, abnormal blood clotting times or cell blood counts, and uncooperative patients. All patients completed a questionnaire to determine whether they had a systemic disease that might affect bone healing, and they underwent periapical radiographs to detect any accessory canal.

Intervention: Patients with defects were randomly allocated to three groups .The defects were not necessarily bilateral or in the same patient. Lesion debridement was used in the control group (Group 1). The first treatment group (Group 2) underwent debridement of lesions and placement of collagenous endomembrane. The second treatment group (Group 3) underwent debridement, application of PRGF gel, and placement of collagenous endomembrane. Surgery was performed on different lesions, regardless of whether they were bilateral or unilateral or in the same patient or different patients. While the initial aim was for an equal number of defects in each group, the final sample comprised a control group with seven lesions, a first treatment group with six lesions, and a second treatment group with seven.

Clinical parameters: These parameters were: VCAL (distance between the CEJ [cemento-enamel junction] to the gingival sulcus), VPD (distance between the free gingival margin to the gingival sulcus), and GI (Silness and Loe index). [12]

Preoperative preparation: Intrabony defects were detected by panoramic radiography, and were more accurately detected after flap reflection using a surgical curette. Clinical parameters were measured preoperatively and 6 months post-surgery using a Williams probe (Hu-friedy-Chicago IL, USA).

Preparation of PRGF: A few minutes before surgery, 20ml of blood was drawn from each patient and transferred to 5ml test tubes containing 3.8% sodium citrate as anticoagulant. The tubes were centrifuged at 460 rpm for 8 minutes in a digital device (PRGF-Endoret Technology System IV, BTI Biotechnology Institute, Minano, Alva, Spain). The separated samples comprised: layer 1, containing 1ml of plasma containing small amounts of growth factor; layer 2 (PGF layer), containing almost twice as much as layer 1 accumulation of growth factors; layer 3 (PRGF layer), containing 0.5ml plasma containing various growth factors; layer 4 (buffy coat layer) containing 0.5 ml of white blood cells; layer 5, containing red blood cells.

Layers 1 and 2 were separated with a 500-microliter pipette. Layer 3 (PRGF) was separated using a 100-microliter pipette in five small aliquots, to prevent contamination from layer 4. Then, 50-microliter calcium chloride (10%) was added for every ml of PRGF to activate the growth-factor-rich plasma.

Surgical procedure: Patients were anaesthetized with lidocaine 2% containing 1:80000 epinephrine using an infiltration technique. Intrasulcular incision was followed by full-thickness flap reflection, debridement of granulation tissue, and scaling and root planning. Without changing the intrabony architecture, the lesions were rinsed with normal saline and the treatment was carried out. All lesions were free from furcation involvement. All 20 lesions were undergone surgical flap procedures. Group 1 then received debridement only. Group 2 underwent debridement and placement of a 2x2 mm2 bioresorbable non-cross-linked collagenous cenomembrane (TRC cenomembrane and collagen pad, Diomem, Tehran, Iran), which covered the lesions, extending by 1mm beyond the defect margins. In group 3, lesions were fully filled with PRGF gel and covered

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in the same manner by membrane. Flap adaptation and suturing were performed with 3-0 Vicryl suture (SUPA, Tehran, Iran). All procedures were carried out by the same surgeon.

Postsurgical procedures: Patients were instructed to use 0.12% chlorhexidine mouth rinse twice daily for 4 weeks, and were prescribed with 400mg ibuprofen and 500mg amoxicillin three times daily for 7 days. They were followed-up for 8 weeks, attending once a week for teeth polishing with a rubber cap. In the maintenance sessions, any supragingival plaque was removed and oral hygiene instructions were reinforced. After 6 months post-surgery, the patients returned for measurement of the clinical parameters.

Radiographic procedures:

The first images were taken under standard conditions using a parallel technique with a digital size 2 PSP sensor (Soredex, Helsinki, Finland) and RinnDentsply film holder (Ltd, Weybridge, England, UK). A perforated bite block was filled with duralay impression material (Reliance Dental Mfg company, Chicago, USA) to record the occlusion. After scanning using PCT (Soredex, Helsinki, Finland) and the DFW 2.5(Digora for windows) program, radiographic images were processed, and stored on the computer. The same bite block was used pre- and post-surgery to ensure the same vertical and horizontal angulations were imaged. A size 2 phosphor plate was used, with similar KVP [Kilovoltage peak], mA [milliamperage] and exposure time as the first series. The initial and post-treatment images were digitally subtracted in Photoshop CS6 [Adobe Photoshop cc/2015.5.0 Release, San Jose, California]

Statistical analysis: Results were recorded in mean ± standard deviation (median) format. Kruskal-Wallis and Wilcoxon signed ranks tests were used for analysis of quantitative variables. For qualitative variables, Chi-square test was used. Data were analyzed using SPSS23 (SPSS-Chicago,USA) with a two-tailed statistically meaningful range of $P<0.05$.

Results

A total of 20 intrabony lesions were examined (7, 6 and 7 in the control group, first treatment group and third [PRGF] group, respectively). The control group comprised 2 male and 2 female (age 42.85±6.16 years), who underwent debridement only; the second group, 1 man and 2 women (45.72±3.13 years), who underwent debridement and placement of a collagenous cenomembrane; the third group was 2 women (41.4±1.1 years) who underwent debridement, placement of PRGF gel and the same membrane. Intra-group comparison of PPD demonstrated a significant decrease in all three groups before and after surgery ($P<0.001$) However, inter-group comparison of PPD revealed no correlation either before or after surgery. (Table 1)

Gingival score increases by one score in all groups.

Intra-group comparison of CAL demonstrated significant increase in all groups before and after surgery ($P<0.05$) (Table 2). However, inter-group comparison of CAL revealed no correlation whether before or after. A statistically significant difference was found between radiographic indices in the groups post-surgery ($P=0.009$) (Table 3).

Table 1. Mean pocket depth (PPD) before and 6 months after surgery

Groups	Pre-treatment PPD (mm)	Post-treatment PPD (mm)	P-value**	PPD difference (DIFF¥)
1(n=56)	2.25±1.60 (2.00)	1.89±0.88 (2.00)	<0.001	-0.62±0.92 (0.00)
2(n=48)	3.10±2.00 (3.00)	2.00±0.85 (2.00)	<0.001	-1.10±1.41 (-1.0)
3(n=56)	2.84±1.51 (2.00)	2.21±0.75 (2.00)	<0.001	-0.62±1.41 (0.00)
P-value*	0.251	0.069	-	0.090

Numbers in the table are mean ± standard differentiation (median) *Kruskal-Wallis test **Wilcoxon signed ranks test

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Table 2. Mean clinical attachment levels (CLA) before and at 6 months post-surgery.

Groups	Pre-treatment CLA	Post-treatment CLA	P-value**	CLA difference (DIFF[¥])
1(n=12)	2.21±0.80(2.0)	3.21±0.80(3.0)	0.002	1.0±0.67(1.0)
2(n=12)	2.16±0.38(2.0)	2.91±0.28(3.0)	0.003	0.75±0.45(1.0)
3(n=14)	2.21±0.69(2.0)	3.14±0.66(3.0)	<0.001	0.92±0.26(1.0)
P-value*	0.947	0.478	-	0.180

Numbers in the table are mean ± standard differentiation (median) ∗Kruskal-Wallis test ∗∗Wilcoxon signed ranks test

Table 3. Bone changes (radiographic indices) in the three treatment groups

	Frequency of no change in bone (%)	Frequency of bone loss (%)	P-value*
Group 1	6(85.7)	1(14.3)	
Group 2	1(16.7)	5(83.3)	0.009
Group 3	1(14.3)	6(85.7)	

Discussion

Six months after surgery, all groups showed improvements in PPD and CLA, but not GI. There was a reduction in PPD and an increase in CLA in all treatment modalities performed although statistically significant results were achieved in the control group. Previous study have presented that the aggregation of platelets occurs in a small volume of plasma. [13] As a result, PRP contains high concentrations of PDGF and TGF-β; the former initiates periodontal ligament cells, and the latter initiate's osteoblast proliferation and differentiation. It has been shown that PRP can release these growth factors in damaged periodontal sites and influences both wound healing and regeneration. [13]

One of the key stages of PRP preparation is to centrifuge whole blood and separate its components into layers. Weibrich et al.pointed out the low efficiency of single-spin centrifuges,[14] using in this study, and other more recent studies have found that dual-spin centrifuges (e.g. Harvest Tec [Biotech]) are more effective at extracting growth factors . This may be one reason for the lack of bone formation in the present study. In order to fully evaluate the regenerative treatments, we used digital subtraction radiography (DSR). Christgau et al. [15] also used DSR to examine bone density in intrabony lesions. They found a statistically significant increase (P<0.05) in bone density in both treatment GTR and PRGF and control groups.

In the current study, there was a statistically significant difference in radiographic indices between groups, but most of the intrabony lesions in Group 1 and Group 3 showed no change in radiodensity or bone loss. The sample size in our study was small, which may explain the lack of bone formation in the PRGF group (Group 2). In the study by Christago et al. bone formation was seen 12 months post-surgery in a control group, but the difference between the control and treatment groups was not statistically different. The authors concluded that PRGF only accelerates bone maturation in the early months following surgery, and has no significant effect on the final stages of bone formation. [15]

Our evaluations were only conducted at 6 months post-surgery, and lesions were not examined after 12 months, which is significant given that bone formation is only radiographicaly detectable after 6-12 months. In many studies which evaluate the effect of PRGF on bone regeneration, PRGF was used in combination with autografts. This results in transportation of vital cells in the defect, [16-18] and tissue regeneration only occurs in sites with vital cells, affected by signaling molecules such as growth factors. [19] It is assumed that the vital cells surrounding the intrabony lesion provide sufficient cells for the growth factors in PRGF; therefore, no additional bone materials or auto grafts were used. For future studies, it is recommended to evaluate the effect of PRGF in combination with autografts.

The small sample size of 20 intrabony lesions in this study may be the reason of why there was no statistically significant correlation between the groups for measures of CAL and PPD before or after surgery. A larger sample size is recommended for future studies. The DSR technique also has several limitations. The two images to be subtracted should be taken at the same angle (KVP and MA). Further, the technique depends on visual discrimination alone, which may involve observer error. Ravi et al. (2017) conducted a split-mouth randomized controlled clinical trial to study the

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effect of PRGF and GTR in the treatment of intrabony defects in patients with chronic periodontitis. They concluded that this treatment combination, as well as using GTR alone, improved clinical and radiographic parameters in these patients after 6 months. There was no additive effect of PRGF when combined with GTR to treat intrabony defects in patients with chronic periodontitis, in terms of either clinical or radiologic outcomes.[20] In contrast, all the groups in the current study showed improvements in CAL after surgery (P<0.05). Similarly, PPD significantly improved in all three groups before and after surgery (P<0.001), and there was no correlation between groups before or after surgery (P=0.251) (P=0.069). A statistically significant difference was also observed in radiographic indices in all groups (P=0.009). These results indicate that CAL and PPD is improved regardless of the treatment modifications and even simple debrident will improve periodontal conditions.

Conclusion

PRGF resulted in improvements in all examined parameters except for radiographic indices and GI.

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Authors' contributions

Niloofar Jenabian was responsible for the study concept, literature search, clinical study and manuscript preparation. Maryam Bojarpour contributed to the clinical study, data acquisition and manuscript preparation. Soraya Khafri carried out the data analysis and statistics. Sina Haghanifar was involved in the radiographic study.

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