



ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The effect of injectable platelet-rich fibrin use in the initial treatment of chronic periodontitis

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SUMMARY

Introduction/Objective The objective of the study was to investigate whether there are differences in therapeutic effect between initial treatments of chronic periodontitis [scaling and root planning (SRP)] alone and SRP in conjunction with injectable platelet-rich fibrin (I-PRF) application, comparing clinical parameters after three months.

Methods Twenty-four patients with chronic periodontitis who had at least two sites with probing pocket depth (PPD) ≥ 5 mm on contralateral side participated in the study. Using a split-mouth design, the patients were treated with SRP + I-PRF (study group) or SRP only (control group). The clinical parameters, clinical attachment level (CAL), gingival margin level (GML), PPD, bleeding on probing, and plaque index, were recorded on both sides.

Results Compared to baseline, both treatment modalities demonstrated an improvement in investigated clinical parameters. The mean value of CAL was reduced from 1.97 ± 0.75 (0.25–3.31) to 1.07 ± 0.44 (0.12–1.78) in the study group, whereas it decreased from 1.81 ± 0.66 (0.42–2.96) to 1.48 ± 0.55 (0.22–2.30) in the control group. Similarly, the corresponding values for GML and PPD showed statistically significant difference between the groups ($p = 0.040$ and $p = 0.006$, respectively).

Conclusion Regardless the limited number of patients in the study, initial periodontal therapy in conjunction with injectable platelet-rich fibrin proved to display significant improvement in all clinical parameters compared to initial periodontal therapy alone.

Keywords: chronic periodontitis; injectable platelet-rich fibrin; initial treatment

INTRODUCTION

Periodontitis is a chronic multifactorial disease, characterized by the progressive destruction of periodontal supporting tissues. Periodontitis presents an inflammation developed by disorders of the host immune response to the infections caused by periodontopathogens [1]. Chronic periodontitis (CP) represents a form of destructive periodontal disease that is generally characterized by slow progression [2]. The World Workshop on the Classification of Periodontal and Peri-implant Disease and Condition in 2017 agreed that the disease previously described as “chronic” or “aggressive” would be grouped under a category “periodontitis” [3]. Periodontitis was regarded as the sixth most prevalent disease globally in 2010 and it affected approximately 50% of the adult population worldwide in 2014 [4]. Due to its high prevalence it is essential to constantly upgrade periodontal therapy.

The principal goal of the periodontal therapy is to restrain active inflammation during the disease and possibly provide support for the reconstruction of periodontal tissue defects [5]. Initial periodontal therapy, scaling and root planning (SRP) is not frequently resolute

at repairing disease-related defects [6, 7]. The periodontal wound healing after SRP usually induces the development of a long junctional epithelium, which is responsible for frequent recurrence of a periodontal pocket [8]. To enhance the process of regeneration, the adjunctive therapeutic procedures have been added to the conventional therapy since the end of the last century.

Platelets have been applied in dentistry over the past three decades. These autologous regenerative tools are concentrated suspensions of supra-physiological amount of growth factors (GFs) and, when applied locally, can induce soft and hard tissue regeneration [8]. Platelets are important reservoirs of various GFs and cytokines, which are vital in wound repair and homeostasis [8]. The periodontal wound healing process implies a series of cell-to-cell interactions and molecular signals that are primarily mediated by cytokines and GFs. GFs control enhancing collagen production, cell proliferation and differentiation, as well as blood vessel formation [9].

Platelet concentrates have advanced from the first generation, platelet-rich plasma (PRP) to the second generation, platelet-rich fibrin (PRF). PRF, developed by Choukroun et al. [10], enables a scaffold enriched with platelets

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and GFs, as well as leukocytes. The concentrate is generated from a blood harvest without any artificial biochemical modifications and anticoagulants [10]. Previous research has demonstrated that PRF contains a greater amount of GFs than PRP. It induces higher fibroblast migration and expression of the transforming growth factor- β 1, the platelet-derived growth factor, and the vascular endothelial growth factor [11]. Along with these factors, there is a higher concentration of the fibroblast growth factor, the insulin-like growth factor-1, the epidermal growth factor, and the platelet-derived epidermal growth factor. Thus, they ensure a better environment for regeneration and repair of the defects. Currently, PRF is widely utilized in the surgical treatment of periodontal intrabony defects, treatment of furcation defects, sinus lift procedures, and tissue engineering [12].

Since the standard PRF is not entirely appropriate for injection, a new injectable formulation of PRF (termed I-PRF) enables easier use of the platelet concentrate in a liquid state. After being generated during centrifugation, it maintains its liquid viscosity for about 15 minutes. [13, 14]. Initially, the PRF has been developed at high centrifugation speeds, enabling a formation of a fibrin clot, which could be utilized as a three-dimensional scaffold for the promotion of periodontal regeneration [15].

Generally, the assessment of periodontal therapy consists of a full-mouth periodontal examination, which enables estimation of the degree of tissue inflammation and destruction. This is conducted by objective measuring of clinical attachment level (CAL), gingival margin level (GML), probing pocket depth (PPD), bleeding on probing (BOP), plaque index (PI), and radiographs assessing the alveolar bone level [16].

So far, patients with CP have not been treated with I-PRF during SRP treatment. Therefore, the aim of this study was to determine the effects of local I-PRF application in conjunction with SRP, compared to application of SRP alone, on periodontal clinical parameters of CP.

METHODS

The randomized, split-mouth, controlled clinical trial recruited patients with CP from the Department of Periodontology, School of Dental Medicine, University of Belgrade. The trial evaluated clinical periodontal outcomes after the initial treatment with or without conjunction of I-PRF. This trial had been approved by the Ethics Committee of the Department of Periodontology, School of Dental Medicine, University of Belgrade. After being informed of the research methods, all the patients submitted their written consent for sharing their personal data and their participation in the study. The study was registered at ClinicalTrials.gov as NCT02898675 on September 12, 2016.

For three months, 30 adult patients were included in the study. The preconditions for participating in the study were a presence of minimum 3 mm CAL and horizontal bone loss of both quadrants of the mandible or maxilla, which were confirmed by full-mouth radiograph images.

The following criteria were used in the patient selection:

- Inclusion criteria: age of 20–75 years; a minimum of six teeth per quadrant; a minimum of two teeth in each quadrant with a probing depth \geq 5 mm; BOP had to be at \geq 40% tooth sites; no involvement of furcation; good general health;
- Exclusion criteria: periodontal therapy within the last 12 months; having surgical therapy; use of antibiotics over the last six months; ongoing drug therapy that might have an impact on the clinical signs and symptoms of periodontitis; pregnancy or nursing; current and former smokers.

Clinical charting

Clinical charting was performed immediately before the first treatment. The following examinations were carried out after three months. The research included the examination of all teeth and tooth sites, except the third molars and the tooth sites associated with furcation involvements of degree II and III [17]. The following variables were recorded from the mesio-buccal, mid-buccal, disto-buccal, disto-lingual, mid-lingual, and mesio-lingual surfaces of each tooth: CAL, GML, PPD, BOP, and PI.

The examiner, a specialist of periodontology, performed and noted all the examinations. Prior to the start of the study, the examiner gained the adequate level of competence and reproducibility skills in accordance with the various clinical parameters and indices that were going to be utilized [18].

Treatment procedures

In regard to screening examination, the patients were thoroughly informed on self-performed plaque control activities consisting of using the modified Bass brushing technique, a soft toothbrush, regular toothpaste twice a day, and inter-dental cleaning with inter-dental brushes once a day. A full-mouth SRP was conducted in all diseased sites by using local anesthesia, in one or two sessions, during the period of 24 hours. The standard of oral hygiene was checked at the baseline examination and during the recall visit after three months following the baseline treatment, and further instructions were provided when it was necessary. Three months following the completion of the baseline treatment, all the patients were recalled for professional supragingival plaque control and reinforcement of oral hygiene. Additionally, re-instrumentation was conducted by using the ultrasonic device in all the sites with a remaining PPD of \geq 5 mm.

Preparation of I-PRF

Blood samples were taken into two 10 ml tubes and prepared for I-PRF preparation. The blood without anticoagulant was then centrifuged at 700 rpm for three minutes (60 g) at room temperature by a Duo Centrifuge (Process for PRF, Nice, France). The upper liquid layer was taken as I-PRF by using a syringe. Afterwards, by applying I-PRF into

periodontal pockets through perforations at the point of interdental space on individually formed occlusal splints, it was enabled to hold it there for a longer time. The I-PRF was applied in one quadrant (study group) of the chosen jaw (mandible or maxilla), whereas the physiological saline was inserted in the opposite side (control group). The splint was removed after 15 minutes. Treatment allocation was decided by a toss of a coin.

Statistical analysis

Mean values and standard deviation were calculated. The Mann-Whitney U-test was performed to determine whether the two groups had similar clinical measurements at baseline and whether one treatment produced better clinical results after a three-month follow-up. The Wilcoxon signed-rank test was used to analyze whether clinical measurements differed before and after treatment. For the whole statistical analysis, a significance level of 5% was used. Software package PASW Statistics Version 18.0 (SPSS Inc., Chicago, IL, USA) was used for all calculations.

RESULTS

All the patients' tooth sites did not display any clinical signs of deterioration after a three-month period. It proved to be uneventful healing, without any pain or any other discomfort in either of the treatment modalities. The only discomfort was experienced by three patients, due to repeated blood collection after failing to find an appropriate blood vessel. During the therapy, one patient no longer participated in the study since she got pregnant, and another one left the country. The remaining 24 subjects, i.e. 10 men and 14 women, finished the treatment protocol. The mean age was 37.29 ± 10.23 years, ranging 22–64 years.

At baseline, none of the assessed clinical parameters showed a statistically significant difference between the study and control groups (Table 1).

Throughout the study, a significant gain in CAL, GML, BOP, PI, and a significant reduction in PPD took place in the study group (Table 2), as well as in the control group (Table 3).

Three months after the therapy (Table 4), the mean value of CAL decreased from 1.97 ± 0.75 (0.25–3.31) to 1.07 ± 0.44 (0.12–1.78) in the study group, whereas it decreased from 1.81 ± 0.66 (0.42–2.96) to 1.48 ± 0.55 (0.22–2.3) in the control group. Similarly, the corresponding values for GML and PPD showed statistically significant difference between the groups ($p = 0.040$ and $p = 0.006$, respectively). The major difference was recorded with BOP – at the baseline examination, 57% of the surfaces in the study group and 61% of the surfaces in the control group showed BOP. After a three-month period, a marked improvement in the bleeding scores took place in both groups, so that 15% of the PDT group and 33% of the SRP group had positive scores ($p = 0.00$). Initially, PI was 0.61 ± 0.517 and 0.64 ± 0.19 , respectively. After three months, plaque values were markedly reduced, and no statistically significant

Table 1. The mean values of clinical parameters of both groups at baseline

Index	Study group X ± SD (min–max)	Control group X ± SD (min–max)	^a p
CAL	1.97 ± 0.75 (0.25–3.31)	1.81 ± 0.66 (0.42–2.96)	0.404
GML	1.72 ± 0.6 (0.02–2.5)	1.86 ± 0.56 (0.75–2.54)	0.457
PPD	3.68 ± 0.72 (1.63–4.53)	3.68 ± 0.89 (1.67–4.96)	0.975
BOP	0.57 ± 0.21 (0.19–0.96)	0.61 ± 0.17 (0.31–0.94)	0.433
PI	0.61 ± 0.517 (0.29–0.92)	0.64 ± 0.19 (0.31–0.91)	0.413

CAL – clinical attachment level; GML – gingival margin level; PPD – probing pocket depth; BOP – bleeding on probing; PI – plaque index;
^aMann-Whitney test

Table 2. The mean values of clinical parameters of study group at baseline and after three months

Index	Baseline X ± SD (min–max)	After 3 months X ± SD (min–max)	^a p
CAL	1.97 ± 0.75 (0.25–3.31)	1.07 ± 0.44 (0.12–1.78)	0.000*
GML	1.72 ± 0.6 (0.02–2.5)	0.62 ± 0.49 (-0.72–1.3)	0.000*
PPD	3.68 ± 0.72 (1.63–4.53)	1.73 ± 0.64 (1.03–2.98)	0.000*
BOP	0.57 ± 0.21 (0.19–0.96)	0.15 ± 0.18 (0–0.9)	0.000*
PI	0.61 ± 0.517 (0.29–0.92)	0.19 ± 0.23 (0–1.15)	0.000*

CAL – clinical attachment level; GML – gingival margin level; PPD – probing pocket depth; BOP – bleeding on probing; PI – plaque index;

^aANOVA;

*statistically significant

Table 3. The mean values of clinical parameters of both groups after three months

Index	Study group X ± SD (min–max)	Control group X ± SD (min–max)	^a p
CAL	1.07 ± 0.44 (0.12–1.78)	1.48 ± 0.55 (0.22–2.3)	0.003*
GML	0.62 ± 0.49 (-0.72–1.3)	0.99 ± 0.57 (0.12–2.1)	0.040*
PPD	1.73 ± 0.64 (1.03–2.98)	2.31 ± 0.73 (1.22–3.58)	0.006*
BOP	0.15 ± 0.18 (0–0.9)	0.33 ± 0.12 (0–0.58)	0.000*
PI	0.19 ± 0.23 (0–1.15)	0.2 ± 0.89 (0.12–0.5)	0.112

CAL – clinical attachment level; GML – gingival margin level; PPD – probing pocket depth; BOP – bleeding on probing; PI – plaque index;

^aMann-Whitney test

Table 4. The mean values of clinical parameters of control group at baseline and after three months

Index	Baseline X ± SD (min–max)	After 3 months X ± SD (min–max)	^a p
CAL	1.81 ± 0.66 (0.42–2.96)	1.48 ± 0.55 (0.22–2.3)	0.000*
GML	1.86 ± 0.56 (0.75–2.54)	0.99 ± 0.57 (0.12–2.1)	0.000*
PPD	3.68 ± 0.89 (1.67–4.96)	2.31 ± 0.73 (1.22–3.58)	0.000*
BOP	0.61 ± 0.17 (0.31–0.94)	0.33 ± 0.12 (0–0.58)	0.000*
PI	0.64 ± 0.19 (0.31–0.91)	0.20 ± 0.89 (0.12–0.5)	0.000*

CAL – clinical attachment level; GML – gingival margin level; PPD – probing pocket depth; BOP – bleeding on probing; PI – plaque index;

^aANOVA;

*statistically significant

differences were recorded between plaque scores of surfaces treated by both therapy modalities ($p = 0.012$).

DISCUSSION

Obviously, the initial treatment of CP aims to achieve the results that can ensure a long-term improvement in clinically measured parameters. This randomized clinical trial with a split-mouth design displayed the difference between the effects of SRP in conjunction with I-PRF vs. SRP alone

in terms of changing clinical periodontal outcomes during the initial treatment of CP.

The obtained results demonstrated that both therapeutic modalities could result in statistically significant improvement of all explored clinical parameters three months after initiating the therapy. At baseline, no significant differences in terms of PPD and CAL were recorded between the two groups. The positive clinical outcomes of the control group after three months correspond with the previous findings concerning clinical efficacy of SRP in treatment of CP. This indicates that in subjects with CP, SRP was successful in reducing PPD and improving CAL [19]. All the patients were trained to maintain oral hygiene regularly. This might have improved the clinical parameters in both groups throughout the study period.

Over the years, the conventional therapy of periodontitis (SRP) has been enhanced by using various adjunctive therapies, mostly by systemically or locally administered antibiotics and antiseptics [20]. Since their use involves some risk, they should be prescribed only for specific situations under optimal conditions. Although the influence of nonsurgical use of lasers on the initial treatment of CP has been considered recently, some studies have shown that its impact on PPD and CAL reduction is less effective than that of antibiotics [20].

Our research is currently focused on novel adjunctive regenerative methods of CP treatment. Although a liquid, injectable form of this platelet concentrate was discovered in 2006 by Choukroun, only the PRF in the form of fibrin membrane was applied during the surgical therapy of CP. For the first time, in this study we tried to adequately use the injectable form of PRF (I-PRF) for a non-surgical treatment of CP. I-PRF is suitable for periodontal pocket application due to its advantage of being in a liquid form.

The injectable form of PRF preparation is based on a slower and shorter centrifugation spin. Moreover, this protocol of centrifugation leads to a higher presence of regenerative cells with higher concentration of GFs, and cytokines, which together may enhance the healing potential of both bone and soft tissues [21].

Clinical trials use CAL to examine various therapeutic modalities that could either reduce the progression of periodontal disease or enable the regeneration of supporting structures. In our study, the progress was made in reducing CAL in the test group more than in the control group three months after the initial treatment ($p < 0.05$). The reduction matches the previous systematic reviews on SRP with different adjuncts, showing that a three-month therapy leads to the CAL value ranging 0.08–1 mm [22, 23]. Our results demonstrated CAL gain by as much as 0.9 mm, representing better outcome compared to the control group with only 0.33 mm reduction. CAL gain during SRP with I-PRF was far higher when compared to SRP alone.

The greater clinical value of CAL gain may be due to more rapid wound healing, less short-term gingival inflammation, and sustained reduction of periopathogenic

bacteria [24]. A study by Dohan et al. [25] shows that I-PRF contains more GFs than PRF, which is six to seven times more loaded with GFs than PRF. In addition, those GFs are released steadily within 21 days [11]. The process is enabled due to the fact that after a short period of time, approximately 15 minutes, I-PRF is formed into a matrix scaffold [11]. The scaffold was proved to have a direct impact on the ability of human gingival fibroblasts to migrate, proliferate, release additional GFs and periodontal ligament cell growth, as well as to increase the differentiation of osteoblasts [26]. By preventing the down-growth of junctional epithelium to the root surfaces and suppressing its interference between the root and soft tissue, a new attachment on root surfaces can be formed.

Furthermore, antimicrobial and anti-inflammatory effects of PRF have also been described [27]. Dohan et al. [28] stated that PRF has immunological and antibacterial properties due to its leukocyte degranulation, and possess some cytokines that may induce angiogenesis and pro/anti-inflammatory reactions. The decrease of microorganism concentration in this area results in reducing inflammation. Reducing the inflammation level brings about the decrease of PPD, GML, and BOP values. The study of Van der Weijden and Timmerman [19] reported the mean PPD reductions ranging 1.29–2.16 mm during CP therapy with SRP alone. In our research, PPD in the study group was reduced by 1.95 mm after a three-month period, while the control group showed a significantly lower decrease ($p < 0.05$). At the same time, GML values in the both groups were reduced by 1.1 mm and 0.87 mm, respectively.

BOP was also reduced in both groups after three months. A successful treatment of CP implies a minimal number of sites with BOP ($< 10\%$), with no probing depths ≤ 3 mm [29]. Our results displayed that BOP after SRP alone dropped to 33%, while it decreased to 15% after SRP + I-PRF. It is probably due to the presence of residual pockets deeper than 4 mm. BOP is proved to be a useful prognostic indicator in estimating periodontal tissue after a non-surgical therapy according to sensitivity and predictability calculations. This is further documented by the fact that presence of residual PPD ≥ 6 mm in combination with BOP $\geq 30\%$ was significantly associated with tooth loss [30].

Both groups in our study demonstrated reduced PI after three months and the improvement in oral hygiene.

CONCLUSION

Regardless of the limited number of patients, the results of the present study indicated that local application of I-PRF in conjunction with SRP, compared to SRP alone, had significant effect on periodontal clinical parameters in the treatment of CP.

Conflict of interest: None declared.

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Ефекти примене инјектабилног фибрина богатог тромбоцитима у иницијалној терапији хроничног пародонтитиса

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САЖЕТАК

Увод/Циљ Циљ овог истраживања био је да се утврди да ли постоји разлика у исходу иницијалне терапије хроничног пародонтитиса, на основу клиничких пародонталних параметара после три месеца, применом додатне апликације инјектабилног фибрина богатог тромбоцитима.

Метод У студију су укључена двадесет четири болесника са хроничним пародонтитисом који имају бар у две регије на конралатералним странама вилице дубину сондирања већу од 5 mm.

Употребом методе „подељених уста“, болесници су третирани иницијалном терапијом хроничног пародонтитиса у комбинацији са инјектабилним фибрином богатим тромбоцитима (студијска група) или само иницијалном терапијом хроничног пародонтитиса (контролна група). Клинички параметри – ниво припојног епитела, ниво ивице гингиве, дубина сондирања, крварење на провокацију и индекс плака бележени су са обе стране.

Резултати У поређењу са почетним мерењима, оба терапијска облика су показала напредак у резултатима. Средња вредност нивоа припојног епитела се смањила са $1,97 \pm 0,75$ (0,25–3,31) на $1,07 \pm 0,44$ (0,12–1,78) у студијској групи, док је у контролној групи опала са $1,81 \pm 0,66$ (0,42–2,96) на $1,48 \pm 0,55$ (0,22–2,30). Слично томе, одговарајуће вредности нивоа ивице гингиве и дубине сондирања показале су статистички значајну разлику између група ($p = 0,040$ и $p = 0,0069$).

Закључак Без обзира на ограничени број болесника, иницијална терапија пародонтитиса у комбинацији са инјектабилним фибрином богатим тромбоцитима показује значајно побољшање клиничких параметара у односу на изоловану иницијалну терапију.

Кључне речи: хронични пародонтитис; инјектабилни фибрин богат тромбоцитима; иницијална терапија