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

Ефекти примене инјектабилног фибрина богатог тромбоцитима у иницијалној терапији хроничног пародонтитиса

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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 (SRP) alone and SRP in conjunction with injectable platelet-rich fibrin (I-PRF) application, comparing clinical parameters after 3 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), probing pocket depth (PPD), bleeding on probing (BOP), and plaque index (PI), 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

САЖЕТАК

Увод/Циљ Циљ овог истраживања био је да се утврди да ли постоји разлика у исходу иницијалне терапије хроничног пародонтитиса, процењено на основу клиничких пародонталних параметара након 3 месеца, применом додатне апликације инјектабилног фибирина богатог тромбицитима (и-ПРФ).

Методе У студију су укључена двадесет четири пацијента са хроничним пародонтисом који имају бар у две регије на контра латералним странама вилице дубину сондирања већу од (ППД) ≥ 5 мм. Употребом дизајна „подељених уста“, пацијенти су третирани иницијалном терапијом хроничног пародонтитиса (СРП) + И-ПРФ (студијска група) или само СРП (контролна група). Клинички параметри - ниво припојног епитела (ЦАЛ), ниво ивице гингиве (ГМЛ), дубина сондирања (ППД), крварење на провокацију (БОП), и плак индекс (ПИ) бележени су са обе стране.

Резултати У поређењу са почетним мерењима, оба терапијска модалитета су показала напредак у резултатима. Средња вредност ЦАЛ се смањила са 1.97 ± 0.75 (0.25–3.31) на 1.07 ± 0.44 (0.12–1.78) у студијској групи, док је у контролној групи опала са 1.81 ± 0.66 (0.42–2.96) на 1.48 ± 0.55 (0.22–2.30). Слично томе, одговарајуће вредности ГМЛ и ППД показале су статистички сигнификантну разлику између група ($p = 0.040$ и $p = 0.0069$).

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

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

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 about half 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 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 for over the past three decades. These autologous regenerative tools are concentrated suspensions of supra-physiological amount of growth factors (GFs) and, when applied locally, they can induce soft and hard tissue regeneration [8]. Platelets as 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 are in charge of enhancing collagen production, cell proliferation and differentiation, as well as blood vessels formation [9].

Platelet concentrates have advanced from the first generation, platelet rich plasma (PRP) to the second generation, platelet rich fibrin (PRF). Platelet-rich fibrin (PRF), developed by Choukroun et al. enables getting a scaffold enriched with platelets and GFs, and leukocytes as well [10]. The concentrate is generated from a blood harvest without any artificial biochemical modifications and anticoagulants [10]. The previous researches have demonstrated that PRF contains greater amount of GFs than PRP. It induces higher fibroblast migration and expression of a transforming growth factor-β1 (TGF-β1), a platelet-derived growth factor (PDGF), and a vascular endothelial growth factor (VEGF) [11]. Along with these factors, there is higher concentration of a fibroblast growth factor (FGF), an insulin-like growth factor-1 (IGF-1), an epidermal growth factor (EGF,) and a platelet-derived epidermal

growth factor (PDEGF). 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 eassier use of 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 fibrin clot, which could be utilized as a three-dimensional scaffold for 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 an alveolar bone level [16].

So far, patients with chronic periodontitis have not been treated by 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 chronic periodontitis.

METHODS

The randomized, split-mouth, controlled clinical trial recruited patients with chronic periodontitis (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 was approved by the Ethics Committee of Department of Periodontology, School of Dental Medicine, University of Belgrade. After being informed of the research methods, all the patients submitted their written consents for sharing their personal data and their participation in the study. The study was registered at ClinicalTrials.gov as NCT02898675 on September 12th, 2016.

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

The below mentioned criteria were used in the patient selection:

- Inclusion criteria: ages 20 to 75; a minimum of 6 teeth per quadrant; a minimum of 2 teeth in each quadrant with a probing depth (PD) ≥5 mm; bleeding on probing (BOP) had to be at ≥40% tooth sites; no involvement of furcation; good general health.

- Exclusion criteria: periodontal therapy within last 12 months; having surgical therapy; use of antibiotics over the last 6 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 recordings

Clinical recordings were performed immediately before the first treatment. The following examinations were carried out after 3 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 II and III degree (Hamp et al.1975). Following variables were recorded from the mesio-buccal, mid-buccal, disto-buccal, disto-lingual, mid-lingual, and mesio-lingual surfaces of each tooth: clinical attachment level (CAL), gingival margin level (GML), probing pocket depth (PPD), bleeding on probing (BOP), and plaque index (PI).

The examiner, a specialist of periodontology, performed and noted down all the recordings. Prior to the start of the study, the examiner gained the adequate level of competence and reproducibility skills according to the various clinical parameters and indices that were going to be utilized (Polson 1997).

Treatment procedures

As for screening examination, the patients were informed thoroughly 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 recall visit after 3 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 ≥5mm.

Preparation of I-PRF

Blood samples were taken into two 10ml tubes and prepared for I-PRF preparation. The whole blood without anticoagulant was then centrifuged at 700 rpm for 3 min (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 performed by a toss of a coin.

Statistical Analysis

Mean values and SD 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 3-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. A software package SPSS.18 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 both 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 the other one left the country. The remaining 24 subjects, i.e. 10 men and 14 women, finished the whole treatment protocol. The mean age was 37.29±10.23, ranging from 22 to 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 (0.22-2.30) 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). 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 of 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 in both groups respectively. After 3 months, plague values were markedly reduced, and no 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 3 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 after 3 months of the control group correspond the previous findings concerning clinical efficacy of SRP in treatment of CP. It indicated that in subjects with CP, SRP was successful in reducing PPD and improving CAL [17]. All patients were trained to maintain oral hygiene regularly. This might improve 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 [18]. 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 their impact on PPD and CAL reduction is less effective than that of antibiotics [18].

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 composed 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 growth factors, and cytokines, which together may enhance the healing potential of both bone and soft tissues [19].

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 3 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 from 0,08 to 1,0 mm [20,21]. Our results demonstrated CAL gain by even 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 with 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 [22]. The study of Dohan at al. shows that I-PRF contains more growth factors than PRF, which is 6-7 times more loaded with growth factors than PRP [23]. In addition, those growth factors are released steadily within 21 days [11]. The process is enabled because of 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 growth factors and periodontal ligament cell growth, as well as to increase differentiation of osteoblasts [24]. 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 [26]. Dohan et al. 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 [27]. 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 reported the mean PPD reductions ranging from 1.29 mm to 2.16 mm during CP therapy with SRP alone [17]. In our research, PPD in the study group was reduced by 1.95 mm after a three-month period, while the control group showed significantly less 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 [28]. 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 ≥ 6mm in combination with BOP ≥ 30% was significantly associated with tooth loss [29].

Both groups in our study demonstrated reduced PI after 3 months and the improvement in oral hygiene.

CONCLUSION

Regardless 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 chronic periodontitis.

Conflict of interest: None declared.

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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 (0.02–2.50)	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;
[a]Mann–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 (0.02–2.50)	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 (0.00–0.9)	0.000*
PI	0.61 ± 0.517 (0.29–0.92)	0.19 ± 0.23 (0.00–1.15)	0.000*

CAL – clinical attachment level; GML – gingival margin level; PPD – probing pocket depth; BOP – bleeding on probing; PI – plaque index;
[a]ANOVA
*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 (0.22–2.30)	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 (0.00–0.9)	0.33 ± 0.12 (0–0.58)	0.000*
PI	0.19 ± 0.23 (0.00–1.15)	0.20 ± 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;
[a]Mann–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 (0.22–2.30)	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;
[a]ANOVA
*statistically significant

