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THE USE OF INJECTABLE PLASMA RICH FIBRIN (I-PRF) VERSUS SIMVASTATIN GEL IN SURGICAL MANAGEMENT OF INFRA-BONY DEFECTS (A RANDOMIZED CONTROLLED CLINICAL TRIAL)

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ABSTRACT

Objectives: Numerous studies have described the benefits of the injectable plasma rich fibrin (I-PRF) and simvastatin (SMV) gel separately in periodontal regeneration. However, there has been a lack of clinical trials comparing the use of these two agents. The aim of this study was to compare the clinical efficacy of open flap debridement (OFD) either with (I-PRF) or 1.2 % (SMV) gel in the management of patients having infra-bony defects (IBDs).

Subjects and methods: Twenty patients, having stage III grade B periodontitis with (IBDs) were recruited and randomly allocated to either; **Group I (I-PRF)** (n=10) undergoing (OFD) followed by single application of I-PRF, or **Group II (SMV)** (n=10) undergoing (OFD) followed by application of 1.2% simvastatin gel. The following clinical parameters were measured at baseline, and 6 months post-operative; clinical attachment level (CAL), periodontal pocket depth (PD), plaque index (PI) and gingival index (GI), as clinical parameters as well as, defect depth as a radiographic parameter.

Results: Both groups showed significant reduction regarding all clinical parameters with no statistically significant difference between groups at 6 months post-operative. SMV demonstrated a statistically significant higher reduction in defect depth compared to I-PRF.

Conclusion: I-PRF and SMV 1.2% gel as adjunctive local delivery regenerative therapy to (OPD) were effective and comparable clinically. Infrabony defects treated with SMV 1.2% gel showed better bone fill compared to I-PRF radiographically.

KEYWORD: Infrabony defects, I-PRF, Periodontitis, Simvastatin, Stage III grade B.

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INTRODUCTION

Periodontitis is generally defined as chronic immuno-inflammatory disorders affecting the tooth-supporting tissues [1]. It leads to loss of connective tissue attachment and loss of bone around the teeth with the formation of periodontal pockets due to the apical migration of the junctional epithelium [2].

There are many surgical and non-surgical treatment that can help in the therapy of periodontitis. Periodontal pocket debridement and conventional open flap debridement (OFD) were considered the treatment of choice, but it is still insufficient in restoring the destroyed tissues by periodontal diseases [3]. Thus, regenerative therapy aims to compensate for the loss of bone in periodontal pockets and restore morphology and function of the periodontium [4].

Platelet concentrates have been used worldwide in the treatment of many periodontal defects, they were considered as an effective and realistic approach for periodontal regeneration as they release polypeptide growth factors, and have the ability to regulate: cell proliferation, and differentiation and chemotaxis [5]

Among types of platelets concentrate that has been introduced is the injectable plasma rich fibrin (I-PRF) [6]. In addition to its content of platelets and leukocytes, it contains stem cells and endothelial cells. It was proposed as treatment option in different periodontal procedures such as treatment of intra-bony defects, and in many types of muco-gingival defects [7].

Statins are hydroxy-methyl-glutaryl co-enzyme-A reductase inhibiting drugs that are now considered as important agents in bone regeneration due to their pleiotropic effects such as anti-inflammatory effect, angiogenesis, antioxidant, and antibacterial properties. They have been reported to aid in osteoblastic differentiation through enhancing bone morphogenic proteins and prevent production of pro-inflammatory mediators and matrix metalloproteinases (MMPs) [8, 9].

Simvastatin (SMV) is one of the family members of statins, it has been compared to placebo gel as an adjunctive to periodontal pocket debridement in cases of infrabony defects in periodontitis patient and reported that there is an increase in bone level with SMV when compared to placebo gel [10,11].

Despite the numerous studies describing the benefits of the I-PRF and Statins separately in periodontal regeneration, there has been a lack of clinical trials comparing the use of these two agents.

The main objective of this study was to compare the clinical and radiographic outcomes following open flap debridement combined with either I-PRF or 1.2% simvastatin gel in managing infrabony defects.

SUBJECTS AND METHODS

Study design

This study was designed as randomized controlled comparative single center, 2 parallel arms, assessor blinded, clinical trial. The study consists of two groups, each containing 10 patients: Group I (I-PRF) and Group II (Simvastatin 1.2%). Patients were randomly allocated for intervention using computer generated randomization (www.randomizer.org) in ratio 1:1 and blocks of 2 which was performed by another individual other than the principal investigator and clinical assessment was also performed by an investigator blinded to the type of investigation. Twenty patients, having stage III grade B periodontitis with infrabony defect (IBDs) were recruited from the outpatient clinic of Oral Diagnosis, Oral Medicine and Periodontology at Faculty of Dentistry, Ain Shams University. The purpose of the study was explained to all patients and an informed consent was signed before the conduction of the study. The proposal was presented to the faculty of Dentistry Ain Shams University Research Ethics committee and was approved before

starting the research [Ethical approval number (FD-ASU-REC.758)].

The participant was included or excluded according to the following criteria. Inclusion criteria: Both genders with age range between 25 and 40 years. Systemically free according to the modified Burkett's health history questionnaire, ASA I [12]. Patients with stage III, grade B periodontitis; having probing depth (PD) ≥6 mm and clinical attachment loss (CAL) ≥5 mm. Patient having at least one infrabony defect with vertical bone loss ≥3 mm (distance between cemento-enamel junction (CEJ) and base of the defect as confirmed by preoperative intraoral periapical radiographs using standardized parallel technique and good compliance with the plaque control instructions following initial therapy. Exclusion criteria were using antibiotic, anti-inflammatory, and immunosuppressive therapy in the previous 3 months before the start of trial or during the study [13], patients who have undergone periodontal treatment in the last 6 months, pregnancy or lactation, reported allergy to any type of statins as documented in medical history, subjects who were tobacco or alcohol users and vulnerable group of patients (e.g.: prisoners, handicapped, or decision impaired individuals).

Patients grouping and treatment protocol

Group I (I-PRF): included 10 patients with infrabony defects (IBDs) undergoing open flap debridement (OFD) followed by single application of I-PRF (from 1 to 1.5mm) and was considered the experimental study group. Group II (SMV): included 10 patients with (IBDs) undergoing (OFD) followed by application of 1.2% simvastatin gel (from 1 to 1.5 mm) [14] and was considered the comparative control group.

Treatment protocol

Pre surgical phase: All patients were evaluated clinically by measuring: Plaque index (PI), Gingival index (GI), Pocket depth (PD), Clinical attachment level (CAL) using Michigan O' probe with William's markings and radiographically by assessing the defect depth by using digital intraoral periapical radiographs using long cone paralleling technique. Digital intra-oral periapical radiographs were taken to the patients for selected deepest pockets by using long cone paralleling-angle technique with special film holder (Rinn extension cone paralleling (XCP) device). Digital intra-oral radiographic system Digora optime was used with photostimulable phosphor (PSP) imaging plate as image receptor. Bite block was used to obtain standardized radiographs and a position aiming device.

They also underwent closed mechanical debridement using ultrasonic scaler and hand instruments, followed by proper oral hygiene instructions. After 4-8 weeks all patients were assessed again, and data recorded as baseline data.

Surgical phase: All patients were then subjected to surgical procedure consisting of the reflection of a full thickness flap and open flap debridement using ultrasonic scalers and periodontal curettes to remove all tissue tags and granulation tissue.

Intervention: 1- I-PRF Preparation: for all patients, who received I-PRF, a venous blood sample was collected in plastic tubes and immediately centrifuged using DUO machine for three minutes at 700 rpm, according to *Choukroun* technique. After centrifugation, I-PRF was immediately collected using a 5 mm plastic syringe. 2- Preparation of 1.2% simvastatin gel: A 4% methyl cellulose 4000 centipoise (C*ps)* gel was prepared by dispersing 2 gm of methyl cellulose powder in 50 ml of hot distilled water 50 - 60°C (as the methyl cellulose starts to melt at 65.7°C). 1.2mg of SMV was added to distilled water to produce 1.2% concentration of the drug in the gel [10].

According to the assigned groups, the treatment was applied: -In Group I: I-PRF was applied 15 mins after debridement, when it becomes a gel to be easily applied in the IBD. **Figure (1)** -In Group II: Simvastatin 1.2% gel was applied after debridement

in the IBD. **Figure (2)**. The mucoperiosteal flaps were then repositioned then sutured. All patients received postoperative instruction including rinsing with 0.12% chlorhexidine hydrochloride (twice daily for 1 week), amoxicillin antibiotic with clavulanic acid 1 gm (twice per day every 12 hours) and Diclofenac anti-inflammatory (50 mg per day every 8 hours for 1 week). All patients were re-examined after 14 days for suture removal and all surgeries were performed by the same operator.

After 6 months, all patients were reassessed clinically by measuring (PI, GI, PD and CAL) and radiographically; Bone fill was measured on the radiograph by measuring the difference in vertical distance from the CEJ to the base of the defect as shown in **Figure (3)** as following; 1- Two horizontal lines were drawn; a line at the CEJ and a line at the most radiographically accentuated bone level at the base of defect of the related root. 2- A line was drawn from the CEJ along the root surface parallel to the long axis of the defect related root to the base of the defect. 3- The measurements were performed twice at baseline and 6 months after surgery then the difference between both readings were calculated to measure the bone fill. All the obtained data at 6 months was compared to the measurements taken at baseline.

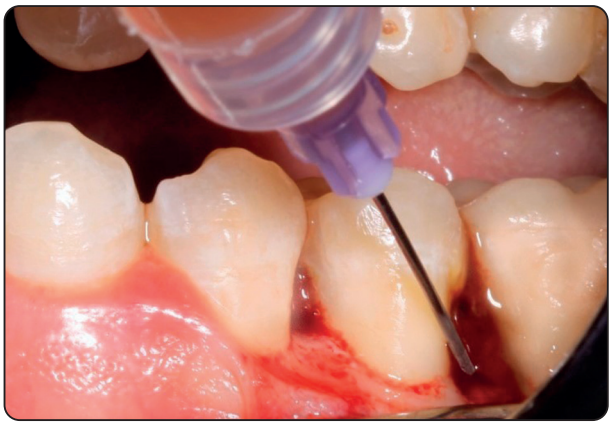

Fig. (1) Application of I-PRF in the IBD (Group I)

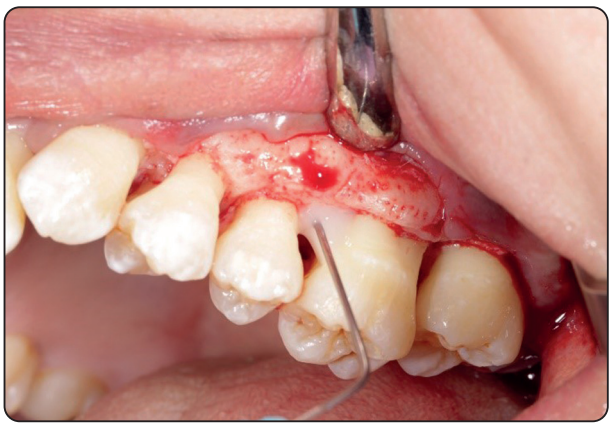

Fig. (2) Application of Simvastatin gel in the IBD (Group II)

Statistical analysis

Numerical data were presented as mean and standard deviation (SD) values. They were explored for normality by checking the data distribution and using Kolmogorov-Smirnov and Shapiro-Wilk tests. Data showed parametric distribution, so they were analyzed using one-way ANOVA followed by Tukey's post hoc test for intergroup comparisons and repeated measures ANOVA followed by main effects comparisons utilizing Bonferroni correction for intragroup comparisons. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with R statistical analysis software version 4.1.1 for Windows.

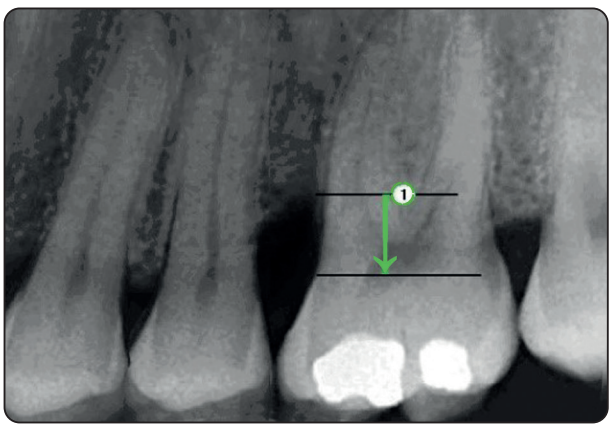

Fig. (3) Baseline radiograph digitized image showing measuring infrabony defect depth.

RESULTS

Demographic data

This study was conducted on 20 cases with stage III grade B periodontitis that were randomly and equally allocated to each of the studied groups: Group I (I-PRF) n=10, Group II (SMV) n=10. The mean age of study population was 32.5±3.25 years with age range of 25-40 years. In both groups, majority of patients were females and most of the treated teeth were found in the upper arch and were molars. There was no significant difference between both groups regarding mean age and sex distribution (p>0.05).

Clinical Outcomes

Regarding the PI and GI assessment at baseline and 6 months post operative, there was no difference in the scoring as well as insignificance between the groups. While during **the periodontal pocket depth** assessment, Group I showed higher reduction in depth after 6 months in comparison to Group II, but still statistically insignificance (p=0.279). By comparing the percentage of change between both groups in probing depth at baseline and at 6 months post operative, it was found that the decrease in Group I was higher than Group II, although this difference was not statistically significant (p=0.267) **Table (1)**. **The clinical attachment level** revealed significant attachment gain at 6 months for both groups with Group II showing higher insignificant gain in comparison to Group I (p=0.695). By comparing the percentage of change between both groups in clinical attachment level at baseline and at 6 months post operative, it was found that the gain in Group II was higher than Group I, although this difference was not statistically significant (p=0.849) **Table (2)**.

Radiographic Outcomes:

The radiographic assessment showed significant bone fill at 6 months for both groups with a significant difference between the groups; showing higher amount of gain for Group II (p<0.001). By comparing the percentage of change between both groups defect depth (bone fill) at baseline and at 6 months post-operative, it was found that the bone fill in Group II was higher than Group I. This difference was statistically significant (p=0.002) **Table (3).**

TABLE (1): Descriptive statistics and test of significance between mean periodontal pocket depth in two groups and the changes by time within each group

Mean deepest periodontal pocket depth (mm)	Group I (I-PRF) (Mean±SD)	Group II (SMV) (Mean±SD)	P-Value
Baseline	6.70±0.67	6.50±0.53	0.470 ns
6 months	3.10±0.32	3.20±0.42	0.556 ns
P-Value	<0.001*	<0.001*	
Mean difference	3.60±0.52	-3.30±0.67	0.279 ns
Percentage change (%)	-53.57±3.76	-50.48±7.68	0.267 ns

; significant (p ≤ 0.05), ns; non-significant (p>0.05)

TABLE (2): Descriptive statistics and test of significance difference between Clinical Attachment Loss (CAL) in two groups and the changes by time within each group

Mean Clinical Attachment Loss (mm)	Group I (I-PRF) (Mean±SD)	Group II (SMV) (Mean±SD)	P-Value
Baseline	5.85±0.58	5.90±0.61	0.854 ns
6 months	3.10±0.70	3.05±0.60	0.866 ns
P-Value	<0.001*	<0.001*	
Mean difference	-2.75±0.42	-2.85±0.67	0.695 ns
Percentage change (%)	-47.42±8.93	-48.22±9.49	0.849 ns

; significant (p ≤ 0.05), ns; non-significant (p>0.05)

TABLE (3): Descriptive statistics and test of radiographic bone fill between two groups and the changes by time within each group

Mean radiographic defect depth (bone fill/mm)	Group I (I-PRF) (Mean±SD)	Group II (SMV) (Mean±SD)	P-Value
Baseline	4.24±0.85	4.23±0.73	0.967
6 months	3.26±0.82	3.02±0.74	0.502
P-Value	0.016*	0.002*	
Mean difference	-0.99±0.10	-1.22±0.06	<0.001*
Percentage change (%)	-24.03±4.43	-29.50±5.18	0.021*

; significant (p ≤ 0.05) ns; non-significant (p>0.05)

DISCUSSION

The present study was designed to compare the effect of simvastatin in comparison with injectable-plasma rich fibrin (I-PRF) in periodontal regeneration by comparing the clinical and the radiographic outcomes following open flap debridement combined with either I-PRF or Simvastatin 1.2% gel locally delivered in infrabony defects.

I-PRF was found to have a potential to enhance intrinsic tissue regeneration for infrabony defects by inducing human mesenchymal stem cells (MSCs) proliferation and migration, and by triggering osteogenic differentiation of MSCs. I-PRF has also been reported to have an anti-inflammatory and anti-microbial activity against many pathogens, which can contribute to faster tissue regeneration [15]

I-PRF was also considered to have the higher concentrations of regenerative cells and growth factors compared to other platelet rich fibrins because of reduced centrifugation speed [16]. Additionally, I-PRF is enriched with interleukin 10 (IL-10), a cytokine involved in reducing inflammatory mediators and prompting tissue regeneration [17]. Finally, this liquid formulation of platelet concentrate offers successful clinical applicability for the clinicians to readily apply the biomaterial alone or in combination with other biomaterials in order to promote bone regeneration [18,19]

Simvastatin was also proved to have an anti-inflammatory effect by inhibiting expression of many inflammatory cytokines such as IL-6, IL-8, IL-1 β and TNF-α level [20] and have an osteo-promotive effect by inhibiting receptor activator of nuclear factor ϰB (RANK), RANK ligand (RANKL), promote the level of osteoprotegerin (OPG) and encouraging differentiation of osteoblasts and promoting neovascularization through its effect on bone morphogenetic proteins and endothelial growth factor [21]. So, it could promote regeneration of bone and healing of soft tissues [22].

According to the obtained results, although PI decreased, the results were insignificant for both groups.

Records of **plaque index (PI)** before and at the end of the study demonstrated that patients were kept under a strict maintenance program, and the overall plaque accumulation was minimal.

Also ,this could be attributed to the anti-inflammatory and antibacterial effect of I-PRF and SMV gel and is similarly coinciding with many studies considering the I-PRF and SMV [15, 16, 17]

Gingival index (GI) showed no statistical difference between both groups after 6 months follow up, this may be attributed to the antimicrobial and anti-inflammatory effects of I- PRF in Group I as stated by *Dohan et al*. 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. [26] Also, in Group II (SMV), the anti-inflammatory properties of simvastatin by decreasing IL-6 and IL-8 production [23]

The statistically significant reduction in probing depth (PD) and clinical attachment level (CAL) that have been observed in both groups could be attrib-

uted to the resolution of inflammation, shrinkage of the gingiva which leads to reduction of pocket depth and clinical attachment gain.

Regarding pocket depth (PD), findings of the present study are in agreement with other studies as [27, 28, 29] ***;*** which all stated that PRF has the ability to induce pocket reduction , and according to the current study the pocket reduction was about (3-3.6 mm) similar to the pocket reduction observed with [28] that was in the range of 3.24–3.35 mm in infrabony defects and also in the study of [29] , the reduction of PD was about 3.15 mm. This slight difference may be due to variation in healing as the two study were done on different populations. On the other hand, a study showed that the reduction of pockets using I-PRF was about 2 mm and this difference may be due to the 3 months follow up and the local delivery of the I-PRF in the pocket [30]***.*** As stated by ***Choukroun et al,*** the high concentration of platelets, leukocytes and growth factors (e.g., platelet derived growth factor, transforming growth factor-b, vascular endothelial growth factor) in I-PRF that are released slowly over time, facilitates regeneration of lost periodontal tissues [24,25,34]

In group II (SMV), our results were in accordance with what was reported by [10,11,27] in variable trials in which all stated that simvastatin has the ability to induce pocket reduction, and according to the current study the pocket reduction was about (3-3.3 mm) similar to the pocket reduction observed with (27) that was (2mm) the slight difference may be due to variation in healing as the two study were done on different populations. As stated by many authors, it can stimulate bone formation by stimulating the production of bone morphogenetic protein-2 (BMP-2) and it promotes the release of vascular endothelial growth factor (VEGF) that stimulate osteoblast differentiation and bone nodule formation [14,38]

Regarding Clinical attachment gain, in group I, the results of our study are in agreement with that of many [31] [29][32] [33] which all stated that PRF reduce the clinical attachment level. In our study the gain was about 3 mm and percent change were about -47 % and this agreed with [29] which have clinical attachment gain about 2.66 mm. This difference may be due to the use of PRF instead of I-PRF which is less in number of growth factors necessary for healing. Another study by [30] stated that the clinical attachment gain is about 0.9 mm after 3 months follow up when I-PRF is used locally in adjunctive with non-surgical periodontal treatment. However, other two studies shows that I-PRF showed no significant attachment gain as [33][34].

Detection of the radiographic bone fill showed that on comparing pre and postoperative of each group revealed statistically significant decrease in defect depth. By comparing both groups to each other, group II (SMV) showed higher reduction in defect size than group I (I-PRF). The results were in accordance with results of study done by [27] in which percentage of bone fill was (25.16%). The current study results could be attributed to the fact that I-PRF contains many growth factors more than any other platelets concentrates with subsequent critical role in bone regeneration in infrabony defects [20,21,22]. Regarding SMV, our results were also supported by the results of other studies as [10,11,37,38,39] which all stated that simvastatin has the ability to induce formation of new bone, and according to the current study the fill was about (1.2 mm) which is different from the bone fill observed with that was (2.15mm), this difference may be attributed to difference in defect type and different population.

SMV gave better outcomes than I-PRF regarding bone fill, this is due to BMP-2 expression, its osteoblastic differentiation potential and stimulation of neovascularization during bone regeneration. In addition, its anti-inflammatory effect, antioxidant effect and angiogenesis play an important role in host modulation which may lead to the decrease in pro-inflammatory cytokines which helped in improving the periodontal treatment outcomes.

Based on the results of the current study and within its limitation, it could be concluded that, although there was no statistically significant difference between both groups in plaque index, gingival index, probing depth and clinical attachment gain but the difference regarding radiographic bone fill was significant higher in Simvastatin group. However, both groups showed bone gain after 6 months.

CONCLUSION

Improvement in clinical periodontal parameters between both groups was comparable. However, infrabony defects treated with SMV 1.2% gel showed better bone fill radiographically in comparison to I-PRF.

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Conflict of interest the authors declare that they have no conflicts of interest in this study.

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