Effect of Locally Injectable Platelet-Rich Plasma As An Adjunct to Scaling and Root Planing on Salivary Mmp-8 Level In Periodontitis Patients

Mohamed A. El-Sharabasy[1], Bassant Mowafey[2], Mohamed M. Anees[3], Azza A. Elbaiomy[4]

Abstract:

Objective: To assess the effect of platelet-rich plasma (PRP) local injection in the treatment of periodontitis, as an adjunct to scaling and root planing (SRP).**Materials and Methods:** Ninety subjects were involved in this study. They were divided into 3 groups. Group I (negative control) included 30 subjects with healthy periodontium. Group II (positive control) included 30 periodontit is patients who were treated by SRP planing. Group III (study) included 30 periodontitis patients who were treated with SRP and PRP injections. Plaque index (PI), Gingival index (GI), probing pocket depth (PPD), and clinical attachment level (CAL) in addition to MMP-8 level recorded at baseline for all groups and 2 months post-treatment for groups II and III .**Results:** At baseline; PI, GI, PPD, CAL, and MMP-8 revealed no significant difference between group II and III ($p>0.05$), while the periodontal parameters of both groups showed highly significantly higher values versus group I ($p<0.001$ for each). Both groups showed a highly significant decrease in PI, GI, PPD, and CAL after treatment ($p<0.001$ for each). In addition, Group III showed significantly lower values in PI ($P=0.028$) and GI ($p=0.032$) and a highly significant decrease in PPD and CAL ($p<0.001$ for each) versus Group II. However, after treatment, both groups II and III showed a highly significant decrease in MMP-8 ($p<0.001$ and $p=0.010$ respectively). No significant difference was found in the MMP-8 level between Group II and III as the mean±SD was 1.73±0.58 and 1.9±0.49 respectively. **Conclusions:** Injection of autogenous PRP in addition to SRP in periodontal pockets can enhance clinical parameters of periodontitis.

Introduction:

Periodontitis is an inflammatory disease of the supporting tissues of teeth.[1] The pathogenesis of periodontal diseases is mediated by the inflammatory response to bacteria in the dental biofilm. There is evidence that specific microbes are associated with the progressive forms of the disease as Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Treponema denticola, and Porphyromonas gingivalis.[2,3] They can create a gathering of destructive factors which can encourage microbial colonization, damage and invasion of periodontal tissue, and interfere with tissue healing.[4]

The target of the management of periodontitis is to reduce infection, resolve inflammation and create a clinical tissue condition, which is compatible with periodontal health.[5] SRP without adjunctive is often sufficient to suppress bacterial pathogens, thereby attaining periodontal health. Adjunctive chemotherapies can be used to improve outcomes at sites not responsive to SRP.[6] The main action of these agents during the healing phase and the active phase of periodontal therapy is preventing supragingival plaque accumulation.[7] Several antibiotics have been tested for clinical and microbiological efficacy in periodontal only a limited number of antimicrobial Agents have been used so far in local delivery formulations.[8]

PRP contains growth factors that influence wound healing, such that it can greatly contribute to tissue repair. PRP reduces bleeding while enhancing soft tissue healing and bone regeneration. Moreover, the cost of regeneration therapy can be reduced using PRP.[9] One of the most studied biomarkers is matrix metalloproteinase-8 (MMP-8). Lee et al. [10], Romanelli et al.[11],Kiili et al.[12],Sorsa et al [13,14], and Alassiri et al.[15] have shown how increased levels of active matrix metalloproteinase-8 (aMMP-8), but not total or latent MMP-8, differentiate periodontitis from gingivitis and precede periodontal attachment loss.

Material and Methods:

Sample size: Sample size calculation was based on MMP-8 Level among periodontitis patients versus control group retrieved from previous research Konopka[16] Using G*power version 3.0.10 to calculate sample size based on effect size of 0.89, 2 tailed test, α error =0.05 and power = 90.0% then total sample size will be 28 in each group at least.

Patient Population: The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Dentistry, Mansoura University, Egypt (Ethical committee approval no.07010720). Written

1Postgraduate MSc student, Department of Oral Medicine, Periodontology, Periodontology, Oral Diagnosis and Radiology Faculty of Dentistry, Mansoura University 35561,Mansoura , Egypt. sharabasy1000@mans.edu.eg
2Lecturer, Department of Diagnosis and Oral Radiology , Faculty of Dentistry, Mansoura University, Egypt.
[3]Prof. of Oral Medicine and Periodontology, Faculty of Dentistry, Mansoura University.
[4]Prof. of Clinical Pathology, Faculty of Medicine, Mansoura University.
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informed consent was obtained from all patients who were recruited for the study. They were informed about the purpose of the study, the treatment received and the steps performed, including the surgical procedures, the possible risks, and possible side effects. Ninety subjects of both sexes, aged between 35-55 years were involved in this study; periodontitis patients with deep isolated periodontal pockets ≥5 mm after successful phase I Therapy and thirty individuals with healthy periodontium. Exclusion criteria included smoking, pregnancy or lactation, history of systemic diseases, and history of antibiotic and periodontal therapy in the last 3 months.

Study design: The participants were assigned to three groups. Group I (negative control) included 30 subjects with healthy periodontium. Group II (positive control) included 30 periodontitis patients who were treated with SRP planing. Group III (study) included 30 periodontitis patients who were treated with SRP and PRP injections. PI, GI, PPD, and CAL in addition to MMP-8 level were recorded at baseline for all groups and 2 months post-treatment for the positive control and study groups. Scaling, root planning, and oral hygiene instructions were provided to periodontitis groups.

Platelet‑rich plasma preparation and application :

Ten milliliters of blood were withdrawn from each patient into five sodium citrate vacuum tubes (each tube with 2 ml), with a gentle inversion several times to ensure the complete mixing of blood with the anticoagulant. The blood was centrifuged for two cycles.[17] The first cycle was at 1000 (rpm) for 5 min, and then, the plasma layer (which contains platelets and white blood cells) was separated into a plain tube ready for the second cycle. The second cycle was at 4000 rpm for 10 min. The upper two-thirds of the solution was discarded and the lower third (which contains platelet pellets) was resuspended. After anesthetizing the affected tooth, root planning was done using Gracey curettes (Zeffiro, Lascod, Italy). Just before injection, 10% of the syringe volume was filled with calcium chloride (activator) and 90% by PRP and added slowly with forth and back motion to mix the syringe contents. The injection was done into the base of the pocket using an insulin syringe.[18]

Saliva sample collection (Swabbing method): the subject was instructed to sit comfortably on a dental Chair and rinse the mouth thoroughly using distilled Water to remove any food debris. A synthetic Cotton pad was introduced into the mouth, at the orifices of major salivary glands. The subject was asked to chew such that the sponge gets soaked in the saliva. The saliva-soaked sponge was removed and placed in a sterile test tube and sent immediately to the lab. Saliva was obtained from the cotton pad by centrifugation and kept frozen till the time of analysis of MMP-8. [19]

Laboratory assessment: Salivary matrix metalloproteinase was assayed by ELISA supplied by Bioassay Technology (China).

Statistical Analysis: The collected data was revised, coded, tabulated, and introduced to a PC using the statistical package for Social Science (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp). Data was presented and suitable analysis was done according to the type of data obtained for each parameter.

Results:

Table 1 shows the clinical assessment of periodontal parameters (PI, GI, PPD, CAL) for all groups at baseline and 2 months after treatment for periodontitis patients. At baseline; PI, GI, PPD, and CAL revealed no significant difference between the positive control (SRP) and the study (SRP+PRP) groups, while the values of both groups showed highly significant higher values versus the clinically healthy group (data not shown). Both the positive control (SRP only) and the study (SRP+PRP) groups showed a highly significant decrease in PI, GI, PPD, and CAL after treatment ($p<0.001$). In addition, the study group showed significantly lower values in PI ($P=0.028$) and GI (0.032), and highly significant lower values in PPD ($p<0.001$), and CAL ($p<0.001$) after treatment versus the positive control group (data not shown).

Table 1: Comparison of periodontal parameters before and after treatment among studied groups

		Group I Negative Control (Healthy)		Group II Positive control (SRP)			Group III Study(SRP+PRP)		
		N=30		N=30		*p*	N=30		*P*
		Mean	±SD	Mean	±SD		mean	±SD	
PI	Before	0.64	0.20	2.61	0.58	<0.001	2.30	0.76	<0.001
	After			1.19	0.13		0.79	0.20	
GI	Before	0.68	0.21	2.54	0.59	<0.001	2.27	0.75	<0.001
	After			0.93	0.25		0.63	0.14	
PPD	Before	3.18	0.62	5.34	0.72	<0.001	5.35	0.72	<0.001
	After			3.68	0.84		1.34	0.38	
CAL	Before	2.30	0.52	5.35	0.71	<0.001	5.31	0.70	<0.001
	After			3.68	0.84		1.29	0.39	
		p value is highly significant at level≤ 0.001 .							

Table 2: Comparison of MMP-8 before and after treatment among studied groups

		Group I Negative Control(Healthy)		Group II Positive control (SRP)			Group III Study (SRP+PRP)		
		N=30		N=30		*p*	N=30		*p*
		Mean	±SD	mean	±SD		Mean	±SD	
MMP-8	Before	2.2	0.4	2.71	0.73	<0.001	2.71	0.87	0.010
	After			1.73	0.58		1.9	0.49	
P-value is highly significant at level ≤0.001.									

Table 2 shows the laboratory assessment of MMP-8 level for all groups at baseline and2 months after treatment for periodontitis groups. At baseline, both the positive control (SRP), as well as study (SRP+PRP) groups, Were significantly associated with higher MMP-8 when compared to the healthy control group (p=0.005, data not shown).

However, MMP-8 did not differ significantly between positive control and study groups (p>0.05, data not shown). After treatment, both the positive control and study groups showed a highly significant decrease in MMP-8 (p<0.001 and p=0.010 respectively). However, no significant differences were found in MMP-8 level percentage change between SRP only and SRP+PRP groups after treatment (p>0.05, data not shown).

Discussion:

Scaling and root planing stays the foundation of nonsurgical periodontal treatment.[20], but some cases present with isolated deep pockets after successful phase I therapy need further treatment than SRP alone. From this concept arise the need for systems of local drug delivery (LDDS) to rise the impact of SRP.[21] Favourable autogenous treatment materials such as blood derivatives e.g.; PRF and PRP.[22] MMP-8, is one of the major collagenases that has a major part in the destruction of connective tissue and alveolar bone in periodontitis.[23] Therefore, the study aimed to assess the effect of locally injectable PRP as an adjunct to SRP on salivary MMP-8 levels in periodontitis patients.

Regarding our results at baseline; PI, GI, PPD, and CAL revealed no significant difference between the positive control (SRP) and the study (SRP+PRP) groups, while there were highly significant differences between both groups versus the clinically healthy group. This can be explained by the tissue damage caused by periodontal pathogens attack showing signs of damage such as periodontal pocket formation, easily bleeding gingiva, surface ulceration and suppuration, alveolar bone destruction and periodontal ligament, tooth mobility and drifting, and finally tooth loss.[24]

However, after two months of treatment regarding the positive control (SRP) group; PI, GI, PPD, and CAL decreased significantly after treatment. This can be due to a critical decrease in bacterial burden located in subgingival and/or periodontal pathogenic organisms following SRP in patients having periodontitis.[20]

Meanwhile, after two months of treatment in the study group (SRP+PRP); PI, GI, PPD, and CAL decreased significantly after treatment. In addition, the study group showed significantly lower values in PI and GI and highly significant lower values in PPD and CAL after treatment versus the positive control group.

These results came in agreement with Roselló-Camps et al.[25]; they found that topical delivery of PRP resulted in statistically significant higher CAL gain than the control groups. Also, Sammartino et al.[26] found a notable reduction in the probing depth and improvement in the probing attachment level in those cases treated with PRP compared with the controls.

Saleem et al.[27]. added that the adjunctive use of PRP in the regenerative treatment of infrabony defects can be considered an affordable technique to get a better CAL gain and PPD reduction in the surgical treatment of periodontal infrabony defects. Kotsovilis et al[28] summarized that clinical applications of PRP in dentistry reported evidence for beneficial effects in the treatment of periodontal defects.

Platelet-rich plasma is defined as a preparation of autologous plasma improved with a platelet concentration higher than that regularly found in entire blood. A great concentration of platelets can supply and release higher amounts of necessary cytokines and growth factors from their alpha granules than physiologic level to give a regenerative stimulus that boosts healing and enhances repair in tissues with low healing capacity.[29]

The results of in vitro study by Kobayashi et al. suggest that PRP promoted gingival fibroblast migration.[30] Anitua et al.[31] found that plasma rich in growth factors exerts positive effects on periodontal ligament fibroblasts, which could be positive for periodontal regeneration. Abdul Ameer et al.[32] added that PRP decreases inflammation and accelerates the healing process. As a result, it will decrease the pocket depth and increase the attachment gain. Mijiritsky et al.[33] concluded that PRP provides more rapid delivery of GFs to the target site as compared to PRF and GCF.

Regarding the laboratory assessment; the MMP-8 level

at baseline did not differ significantly between the positive control group (SRP) and the study group (SRP+PRP). However, both groups were significantly associated with higher MMP-8 when compared to the healthy control group.

The active phase of periodontal disease is identified non-invasively in oral fluids as a pathological elevation of aMMP-8 levels.[14,34]. These results could be attributed to the tissue destruction which occurred in periodontal tissues as a result of the bacterial endotoxins and host defensive mechanisms increasing the level of MMP-8 in periodontitis patients than the healthy subjects.[35]

After two months of treatment, both the positive control (SRP) and the study (SRP+PRP) groups showed a significant decrease in salivary MMP-8 biomarker levels. Buduneli and Kinane[36] and Kim and Kim.[37] also reported that MMP-8 levels decreased significantly 3 months after non-surgical periodontal therapy in periodontitis patients.

Sorsa et al.[14] mentioned that decreased enzyme levels after periodontal therapy suggest the effectiveness of nonsurgical periodontal therapy in reducing the bacterial load in the periodontal environment. The persistence of matrix metalloproteinase-8 at physiologic levels after treatment has been suggested to reflect the onset of the reparative or protective/defensive phase.

Meanwhile, the results of the present study showed that MMP-8 levels after treatment did not differ significantly between the positive control group (SRP) and the study group (SRP+PRP). This may be explained by the high concentration of PRP (90%) used in the present study as Tavassoli-Hojjati et al. [38] concluded that the vitality and proliferation of PDL cells are stimulated by low concentrations of PRP (5%) and are suppressed by high concentrations (50%), suggesting a dose-related response.

Conclusions:

The application of PRP as an adjunctive to SRP in the treatment of periodontitis showed effective clinical enhancement of periodontal parameters, proving its anti-inflammatory effect and regenerative capacity, and could be a cost-effective adjunctive autogenous effective treatment option for periodontitis patients.

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