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Assessment of Transforming Growth Factor-β1 in Gingival Crevicular Fluid around Bony Defects Treated with Platelet Rich Fibrin (PRF) System

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ABSTRACT

Purpose: This study was carried to evaluate and compare the regenerative capacity of PRF mixed with bioactive glass versus PRF mixed with bioactive glass combined with collagen membrane in treatment of intrabony defects. This assessment was based on TGF- β1 concentration in GCF. **Patients and materials**: A sample of 36 intra bony defects were randomaly divided into; Group I: bioactive glass mixed with PRF. Group II: collagen membrane with bioactive glass mixed with PRF. Group III: open flap debridement only. The GCF TGF-β1 was analyzed by ELIZA at base line, one and three months postoperatively and evaluate the efficacy of PRF system in intrabony defects clinically and radiographically at base line, one, three and six months. **Results:** Mean TGF-β1 concentrations were significantly reduced in one and three months post-surgery when compared to baseline. The mean values of TGF-β1 at 3 months was 0.12± 0, 0.4±0.01 and 0.11±0.01 in groups I, II, III respectively. There was a statistically significant reduction in periodontal probing depth that was 6±0.97 and gain in CAL 3.87±2.14 mm both in group II after 6 months. There was a highest bone density increase was18±5.84 in group I. **Conclusions:** The assessment of cervical TGF-β1 levels is considered to reflect the ongoing processes in the surrounding periodontal tissues. In addition, utilizing PRF mixed with bioactive glass might be considered a promising regenerative therapy.

KEYWORDS

Transforming growth factor-β1, gingival crevicular fluid, ELIZA, platelet rich fibrin, Bioactive glass and collagen membrane.

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INTRODUCTION

Periodontitis is a bacterially induced inflammatory disease of the soft and hard tissues which support the tooth root [1]. The ultimate goal of regenerative periodontal therapy is to prevent further attachment loss and restore the supporting structures (i.e., alveolar bone, periodontal ligament, and root cementum) that were lost due to trauma or disease in such a way that the architecture and function can be re-established [2].

Besides the collagen having the major extracellular macromolecule of the periodontal connective tissue and is physiologically metabolized by these tissues. It has been also shown to be chemotactic for fibroblasts, acts as a barrier for migrating gingival epithelial cells, serves as a fibrillar scaffold for early vascular and tissue ingrowth, facilitates early wound stabilization and maturation, possess hemostatic properties through its ability to aggregate platelets, very weakly immunogenic, and biocompatible [3].

The bioactive glass immediately interacts with the patient's body fluid and to form a silica- and calcium-rich surface gel that traps cellular and no cellular materials within the gel matrix. Within the matrix, hydroxyapatite crystallize and interpose with mucopolysaccharides, glycoproteins, collagen, and osteocellular materials. With time, the "living" matrix is transformed, remodeled, and replaced by newly formed osseous tissue [4,5].

Platelet-rich fibrin (PRF) described by ***Joseph Choukren*** is a second-generation platelet concentrate. It released growth factors such as platelet-derived growth factor and transforming growth factor could promote periodontal regeneration. PRF also helps in facilitating adhesion and spreading of cells, regulates gene expression of growth factors, growth factor receptors [6].

Among growth factors the effects of transforming growth factor-β1 (TGF- β1) on cell proliferation and the differentiation process indicates that this cytokine may have a significant role in wound healing, tissue remodeling and regeneration. Moreover, TGF- β1 stimulates many cell types, thereby increasing synthesis of extracellular matrix molecules. TGF-β demonstrates significant influence as it modulates collagen matrix in both physiological and pathological conditions such as periodontitis. This cytokine is thought to contribute to the maintenance of tissue integrity, as evidenced by its expression and production at healthy sites as well as at periodontally diseased sites [7].

Thus, the aim of the present study was to evaluate clinically and radiographically the efficacy of PRF system in treatment of intrabony defects based on the assessment of TGF-β1 in GCF around bony defects treated with PRF system.

PATIENTS AND MATERIALS

Study sample: this study was undergone on 12 patients (7 male and 5 female) with 36 intra bony defects, selected consecutively from those referred to the Department of Oral Medicine, Periodontology, Diagnosis and Radiology, Faculty of Dental medicine, (Girls branch); Al- Azhar University, seeking for periodontal treatment.

Criteria for patient selection:

Inclusion criteria:

Selected patients were free from systemic illness, evaluated according to dental modification of the Cornell medical index [8].All patients were diagnosed as having chronic periodontitis. Bilateral and/or multiple defects with probing pocket depth were measured as ≥5 and clinical attachment level ≥ 5 [9].

Exclusion criteria:

Patients with any systemic conditions that may contraindicate surgical therapy, had received any type of periodontal treatment in the past 6 months prior to examination and received antibiotics or

anti-inflammatory therapy in the 6 months prior to examination. Patients that may have any habits that may jeopardize the regeneration process, such as heavy smoking and alcoholism and having occlusal trauma or parafunctional habits and who were not willing to follow our treatment protocol. Pregnant or lactating females.

The study groups: Test Group (I): including 12 intrabony defects that have received bioactive glass mixed with PRF. Collagen Group (II): including 12 intrabony defects that have received collagen membrane with bioactive glass mixed with PRF. Control Group (III): including 12 intrabony defects that have received open flap debridement only.

Each patient first received cause-related therapy consisting of scaling and root planning, motivation, and oral hygiene instructions. Four weeks after the completion of initial therapy, re-evaluation examination was performed.

At baseline clinical parameters Probing pocket depth[10], Clinical attachment level [(10)], Plaque Index[11] and gingival index[11] were immediately preoperatively obtained on the day of the surgery by one examiner, who was blind to the type of treatment. Final parameters were taken 1, 3 and 6 months postoperatively by the same examiner.

Gingival crevicular fluid sampling

Gingival crevicular fluid was collected from the same selected sites for surgery. Filter paper was gently placed for 30 seconds into the crevice until a minimum resistance is felt. Care was taken to avoid mechanical injury. Filter paper was kept frozen at -20˚c until assayed.

Radiographic Assessment:

Indirect digital imaging technique was used preoperative and 6 months postoperative. Radiographic examination was performed for assessment of bone height and density at the defect site at baseline and 6 months postoperatively. The mean of the readings was calculated. Alveolar bone level percentage done by a comparison between the linear measurement at baseline and at 6 months post-surgery was calculated to determine the extent of bone fill after the surgical procedure.

Linear measurements were performed to evaluate the change in the alveolar bone level by line was drawn through the CEJ, another line was drawn perpendicular to the first line to the base of the pocket then the percentage of change in alveolar bone level calculated. Additionally, the measurement of bone density made by line on the side of the tooth was drawn 0.2 mm apart and parallel to the tooth surface. The lines extend from the CEJ (as a reference point) to the apex of the root. The mean value of the readings of three points on the line was calculated to get the bone density and repeated after 6 months.

TGF-β analysis:

The GCF TGF-β1 was analyzed by enzyme linked immunosorbent assay (ELIZA) for quantification of this protein in the GCF samples. Evaluation of periodontal disease progression was evaluated by taking GCF samples at base line, one month and three months postoperatively.

Surgical phase:

Sulcular incisions were applied on the buccal and lingual/palatal aspects of the involved teeth in addition to one tooth mesial and distal to it. Full thickness muco-periosteal flap was then elevated by blunt dissection using a periosteal elevator.

The granulation tissue was then removed and thoroughly debridement was carried out with curettes, to ensure a clean site for incorporation of the graft material and membrane.

Protocol for PRF preparation:

A sample of blood is collected from patient without anticoagulant in 10 ml tubes which are

immediately centrifuged at a rate of 3000 rpm for 10 min. After centrifugation, the resultant product consists of three layers. The top most layer consisting of a cellular PPP (platelet poor plasma), PRF clot in the middle and RBCs at the bottom of the test tube. The fibrin clot obtained after centrifugation is removed from the tube and the attached red blood cells scraped off from it and discarded. Under aseptic condition, a fibrin clot formed in the middle part of the tube was obtained.

In group (I): squeezed PRF mixed with bioactive bone graft settled within a sterile amalgam capsule that was inserted in amalgamator for 10 seconds. The final composite was inserted in the defect, the flap was adapted and used vicryl suture [12].

Concerning group (II) a surgical template was applied and trimmed, collagen membrane was cut according to formed template. After filling the defect with the bone graft, the membrane was adapted over the defect extending 2-3mm apical to the crest of the existing bone, then will be secured to the CEJ of the tooth, with vicryl 4/0 suture. After finishing, the flap was secured with interrupted black silk 4/0 sutures to obtain primary closure**.**

In group (III), open flap debridement was carried out. Thoroughly debridement of any defect had been performed without replacement of any graft material.

RESULTS

[Table1] showed the mean PPD recorded at different time shows the correlation between probing pocket depth (PPD) at various time points and there was a significant reduction in PPD among all intervals (p-value<0.05) throughout the course of the study. The difference in PPD between the Pre-surgery and one month Post-surgery (p-value=0.0066), Pre-surgery and three months Post-surgery (p-value=0.0036), pre- surgery and 6 month Post-surgery (p-value=0.0876) and three month and six months Post-surgery (p-value=0.084) were not significant.

Table 1. *Correlation between Probing Pocket Depth (PPD) at Various Time Points.*

Time points		Mean	S.D	
Base line	GI	6.63	0.74	0.0024 S
	GII	8.25	2.19	
	GIII	5.25	1.16	
1 month	GI	4.75	0.71	0.009 S
	GII	3.88	0.95	
	GIII	3.50	0.53	
3 month	GI	3.88	0.58	0.006 S
	GII	3.13	1.22	
	GIII	2.38	0.44	
6 month	GI	3.13	0.58	0.09 S
	GII	2.25	1.22	
	GIII	2.38	0.44	
Difference between TGF-β1 conc. At base line and 1 month				0.0066
Difference between TGF-β1 conc. At base line and 3 month				0.0036
Difference between TGF-β1 conc. At base line and 6 month				0.0876
Difference between TGF-β1 conc. At 3 and 6 month				0.084

[Table 2] showed the mean CAL recorded at different time. It shows the correlation between clinical attachment level (CAL) at various time points and there was a significant gain in CAL among all intervals (p-value<0.05) throughout the course of the study. The difference in CAL between the Pre-surgery and one month Post-surgery (p-value=0.474), Pre-surgery and three months Post-surgery (p-value=0.469), pre- surgery and 6 month Post-surgery (p-value=0.374) and three month and

six months Post-surgery (p-value=0.095) were not significant.

Table 2. *Correlation between Clinical Attachment Level (CAL) at Various Time Points*

Time points		Mean	S.D	
Base line	GI	4.0	0.74	0.043 S
	GII	4.5	2.19	
	GIII	2.5	1.16	
1 month	GI	2.00	0.71	0.517 N
	GII	1.75	0.95	
	GIII	1.50	0.53	
3 month	GI	1.50	0.58	0.512 N
	GII	1.63	1.22	
	GIII	1.25	0.44	
6 month	GI	0.38	0.58	0.417 N
	GII	0.63	1.22	
	GIII	0.25	0.44	
Difference between TGF-β1 conc. At base line and 1 month				0.474
Difference between TGF-β1 conc. At base line and 3 month				0.469
Difference between TGF-β1 conc. At base line and 6 month				0.374
Difference between TGF-β1 conc. At 3 and 6 month				0.095

[Table 3] showed the mean TGF-β1 levels detected at different time intervals. There was significant reduction in TGF-β1 levels at the pre-surgery, one and three months post-surgery intervals when compared to Baseline levels (p-value<0.05). The difference in TGF-β1 levels between the Pre-surgery and one month Post-surgery (p-value=0.067), Pre-surgery and three months Post-surgery (p-value=0.384) and one month and three months Post-surgery (p-value=0.217) were not significant.

Table 3. *Correlation between TGF-β1 Levels at Various Time Points*.

Time points		Mean	S.D	
Base line	GI	0.31	0.09	0.25 S
	GII	0.17	0.03	
	GIII	0.25	0.03	
1 month	GI	0.24	0.08	0.092 N
	GII	0.16	0.03	
	GIII	0.22	0.01	
3 month	GI	0.19	0.09	0.409 N
	GII	0.13	0.04	
	GIII	0.4	0.04	
Difference between TGF-β1 conc. At base line and 1 month				0.067
Difference between TGF-β1 conc. At base line and 3 month				0.384
Difference between TGF-β1 conc. At 1 and 3 month				0.217

[Table 4] showed in all groups, alveolar bone height decreased by time, to reach the least mean value after 6 months. There is a statistically significant decrease by time in group I (p= 0.00012), group II and III (P<0.0001). At baseline, a greater mean alveolar height was recorded in groups I, II. At 6 months observation time, the greatest mean was recorded in group II.

Table 4. *Correlation between alveolar bone height at Various Time Points*

Time points		Mean	S.D	
Base line	GI	38.5	11.09	0.00012
	GII	38.25	9.25	
	GIII	29.25	9.07	
6 month	GI	19.5	14.15	0.0001
	GII	21.5	9.29	
	GIII	20	9.13	

[Table 5] showed in all groups, alveolar density increased by time, to reach a higher value after 6 months. There is a statistically significant decrease by time in groups I & II (P<0.0001), and group III (p=0.0003). At baseline and at 6 months after treatment, a greater mean alveolar density was recorded in group II.

Table 5. *Correlation between bone density at Various Time Points*

Time points		Mean	S.D	
Base line	GI	28.75	7.46	0.0001
	GII	56.00	2.71	
	GIII	33.75	17.11	
6 month	GI	46.75	13.30	0.0003
	GII	69.25	3.30	
	GIII	50.25	13.94	

DISCUSSION

The primary objectives of therapy for patients with chronic periodontitis aims to stop disease progression and to enhance the regeneration of the damaged tissues. However, many variables responsible for complete regeneration of the periodontium are unknown and research is ongoing in this area [13,14].

Resorbable membranes have been developed in an attempt to overcome some of the disadvantages of non-resorbable membranes. The advantages of resorbable membranes include reduced surgical time and morbidity, less risk of damage to regenerated tissues and better biocompatibility. Thus, the present study utilized collagen membrane in group II [15,16].

Inaddition, PRF when used as a grafting material; it creates an improved space making effect which facilitates cell events that are favorable for periodontal regeneration leading to mineralized tissue formation. PRF also helps in facilitating adhesion and spreading of cells, regulates gene expression of growth factors, growth factor receptors, proteins, and determines the outcome of the cell response to growth factors due to the presence of collagen, fibronectin, elastin, other non-collagenous proteins, and proteoglycan in the extracellular matrix of PRF [17].

Bioactive glass had been used as the bone graft substitutes in this study. After implantation within the bony defect, surface reactions ensure deposition of a calcium phosphate layer when exposed to (body) fluid. Sodium, silica, calcium, and phosphate ions are released from the surface and increase the local pH and osmotic pressure [16]. The surface reactions not only are beneficial for the formation of new bone but also ensure that bioactive glass contains antibacterial properties and potentially promotes angiogenesis [17,18].

Biochemical evaluation was performed by collecting GCF sampling in the present study. GCF components can efficiently serve as potential diagnostic and prognostic markers for the progression of periodontitis. The assessment of cervical TGF-β1 levels is considered to reflect the ongoing processes in the surrounding periodontal tissues, including inflammation, turnover of connective tissue and resorption of alveolar bone [10].

Transforming Growth Factor-β1 has highly diverse biological effects including the chemotactic and the mitogenic activity of gingival and periodontal ligament fibroblasts, and the up regulation of ECM components including collagen, fibronectin, tenascin and proteoglycans. Although, the precise role of TGF-β1 in periodontal wound healing remains unclear, it has been found that it has some degree of clinical efficacy in promoting periodontal regeneration [14].

Radiographic bone measurement is a non-invasive, painless, method to measure bone changes. In the study, Vista scan system was used to monitor the

alveolar bone changes and to measure the treatment outcomes. It has been reported that the radiographic evaluation can be an effective indicator of the outcome of periodontal regenerative therapies the need for surgical re-entry procedure [19].

A decrease in GCF TGF-β1 concentration in all groups by time had been reported. One way analysis of variance revealed that there is a statistically significant decrease by time in all groups, whereas the decrease the mean values of TGF-β1 at 3 months was 0.12± 0, 0.4±0.01 and 0.11±0.01 in groups I, II, III respectively. The significant decrease in GCF TGF-β1 concentrations from Baseline to 3 months post-surgery in this study**.** This finding could be due to the elimination of the microbial factors which causes a gradual decrease in the level of bacterial toxins followed by decline in the production of inflammatory mediators and stimulants as well [13].

The mean reduction in probing depth in group I after 6 months was 3.5±0.16 mm. The combined effect PRF and bioactive glass resulted in excellent outcome in the present study. On the other hand, the mean probing depth reduction in group II was 6±0.97 after 6 months. A similar study was done to showed slightly higher values in combination therapy which was than the bone graft alone. This could be attributed to the proper scaling and root planning, as well as the improvements in self performed oral hygiene measures as a result of participation in this study with periodontal examination scheduled monthly [20,21].

There was a highly significant gain in the clinical attachment level in group I, II and III during the study period. The clinical attachment level gain was 3.62±0.41 mm in group I, 3.87±2.14 mm in group II and 2.25±0.47 mm in group III. The effectiveness of autologous PRF in the treatment of intra bony defects of chronic periodontitis patients and resulted in a greater reduction in pocket depth, more gain in clinical attachment level and greater intra bony defect fill than those sites treated with open flap debridement (OFD) alone [20,21].

The mean bone height gain in group II was 16.75±0.04 mm, these results were analogous to those findings which assessed the radiographic changes in periodontal intra bony defects treated with PRF. They concluded that PRF is an effective treatment modality and resulted in an increased post-operative radiographic alveolar height in the treated defects [19,22].

Regarding bone density, there was a highly statistically significant increase in bone density in group I, II and III at 6 months compared to baseline. It was 18±5.84 in group I, 13.25±0.59 in group II and 16.5±3.17 in group III. It could be suggested that the increase in bone density in group II may be due to the effect of bone induction in the regenerated connective tissue attachment [19,22].

CONCLUSION

The results of the present study showed significant changes in TGF-β1 levels and periodontal parameters as a result of periodontal surgical treatment. Moreover, amalgamation of PRF and bioactive glass was effective for the management of intrabony defects, but long-term randomized clinical trials should be undertaken before reaching final conclusion.

REFERENCES

1. Darveau RP (2010) Periodontitis: a polymicrobial disruption of host homeostasis. Nat Rev Microbial 8(7): 481–90.

2. Kolenbrander PE, Palmer RJ, Jr., Periasamy S, Jakubovics NS (2010) Oral multispecies biofilm development and the key role of cell-cell distance. Nat Rev Microbial 8(7): 471–80.

3. Bottino MC, Thomas V, Schmidt G, Vohra YK, Chu TM, Kowolik MJ, et al. Recent advances in the development of GTR/GBR membranes for periodontal regeneration. A materials perspective. Dent Mater. 2012; 28:703–21.

4. Mellonig JT, Nevins M, and Sanchez R. Evaluation of a bio absorbable physical barrier for guided bone regeneration: part II- material and a bone replacement graft. Int J Periodontics Restorative Dent 1998; 18:129e37.

5. Schepers E, Ducheyne P, Barbier L. Long term clinical evaluation of bioactive glass particles of narrow size range. Bio ceramics 1996; 9:99e102.

6. Jiing-Huei Zhao, Chung-Hung Tsai, Yu-Chao Chang. Management of radicular cysts using platelet-rich fibrin and bioactive glass Journal of the Formosan Medical Association (2014) 113, 470e-76.

7. Mize TW, Sundararaj KP, Leite RS, Huang Y. Increased and correlated expression of connective tissue growth factor and transforming growth factor beta 1 in surgically removed periodontal tissues with chronic periodontitis J Periodontal Res 2015; 50: 315–19

8. Busby, M., Chapple, E., Matthews, R. & Chapple, I.L. (2013) Practitioner evaluation of a novel online integrated oral health and risk assessment tool: a practice pilot. [Erratum appears in Br Dent J. 2013 Sep; 215(5):212]. British Dental Journal 215, 115–20.

9. Kebschull M, Demmer RT, Grun B, Guarnieri P, Pavlidis P, Papapanou PN. Gingival tissue transcriptomes identify distinct periodontitis phenotypes. J Dent Res 2014: 93: 459–68.

10. Silvana P. Barros, Ray Williams, Steven Offenbacher and Thiago Morelli. Gingival crevicular fluid as a source of biomarkers for periodontitis. Periodontology 2000.10 December 2015 10.1111/prd.12107

11. Silness, J. & Loe, H. (1964) Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. Acta Odontologica Scandinavica, 22, 121-35.

12. Sanjeev Kumar Salaria, Simrat Kaur Ghuman, Saurabh Kumar, and Garima Sharma. Management of localized advance loss of periodontal support associated Grade II furcation and intrabony defect in chronic periodontitis patient through amalgamation of platelet-rich fibrin and hydroxyapatite bioactive glass composite granules. 2016 Jul-Sep; 7(3): 405–08.

13. Khalid S. Hassan, Adel S. Alagl. A Comparative Study of Bovine Bone Used Alone and in Combination with Transforming Growth Factor-Beta for the Treatment of Periodontal Osseous Defects in Humans. Saudi Journal of Medicine & Medical Sciences | Vol. 3 | Issue 1 | January 2015 | 33-39

14. Mardegan GP, Shibli JA, Roth LA, Faveri M, Giro G, Bastos MF. Transforming growth factor-b, interleukin-17, and IL-23 gene expression profiles associated with human peri-implantitis. Clin. Oral Impl. Res. 28, 2017, e10–e15. doi: 10.1111/clr.12846.

15. Patino MG, Neiders ME, Andreana S, Noble B, Cohen RE. Collagen as an implantable material in Medicine and Dentistry. J Oral Implantol. 2002; 28:220–5.

16. Zeeshan Sheikh, Javairia Qureshi, Abdullah M, Alshahrani Heba, Nassar Yuichi, Ikeda Michael, Glogauer and Bernhard Ganss. Collagen based barrier membranes for periodontal guided bone regeneration applications Odontology (2017) 105:1–12 DOI 10.1007/s10266-016-0267-

17. Shravanthi Raghav Yajamanya, Anirban Chatterjee, Ahad Hussain, Amita Coutinho, Sushma Das, Shobha Subbaiah. Bioactive glass versus autologous platelet-rich fibrin for treating periodontal intrabony defects: A comparative clinical study. March 07, 2017, IP: 124.124.93.5.

18. D. C. Coraça-Huber, M. Fille, J. Hausdorfer, D. Putzer, and M. Nogler, "Efficacy of antibacterial bioactive glass S53P4 against S. aureus biofilms grown on titanium discs in vitro," Journal of Orthopaedic Research, 2014; 32(1): 175–77

19. Sumedha Srivastava, Pradeep Tandon, Krishna Kumar Gupta, Amitabh Srivastava, Vinod Kumar, and Trilok Shrivastava. A comparative clinico-radiographic study of guided tissue regeneration with bioresorbable membrane and a composite synthetic bone graft for the treatment of periodontal osseous defects J Indian Soc Periodontol. 2015 Jul-Aug; 19(4): 416–23.

20. Sattari M, Fathiyeh A, Gholami F, Darbandi H, Mahdi T, Samani G. Effect of surgical flap on IL-1β and TGF-β concentrations in the gingival crevicular fluid of patients with moderate to severe chronic periodontitis. Iran J Immunol. 2011; 8:20–26.

21. Shimada Y, Tabeta K, SuqitaN and Yoshie H. profiling biomarkers in gingival crevicular fluid using multiplex bead immunoassay. Archives of oral biology, 2013; 58,724-30.

22. Reddy MS. Radiographic methods in the evaluation of periodontal therapy. J periodontal 1992; 63:1078-86.