Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4286 - 4294
Received 25 April 2021; Accepted 08 May 2021.

Expression of Transforming Growth Factor – β In Type II Diabetics With Chronic Periodontitis Following Minimally Invasive Periodontal Surgery Under Loupe magnification

Dr.P.S.G Prakash[1*], Dr.Devapriya Appukuttan[2], Dr.Sangeetha Subramanian[2], Dr.Vanaja Krishna Naik[3], Dr. Bharathi S R[4] , Dr.Dhayanand John Victor[5]

[1]MDS, Professor, Department of Periodontics, SRM Dental College and Hospital, Chennai.
[2]MDS, Reader, Department of Periodontics, SRM Dental College and hospital, Chennai.
3MDS, Academic and Research Faculty, School of Dentistry, Faculty of Medicine and Health, University of Leeds.
4 MDS, Private practice, Tiruchengode.
[5] MDS, Professor and Head of the Department, Department of Periodontics,
SRM Dental College and Hospital, chennai.
[*]Corresponding author: akashpsg@gmail.com

ABSTRACT

AIM: To compare and evaluate the expression of Transforming Growth Factor - β (TGF- β) in the Gingival Crevicular Fluid (GCF)following minimally invasive periodontal flap surgery under macro and microsurgical approach during early wound healing in Type II Diabetics with generalised chronic periodontitis (GCP).**METHODS:** Thirty diabetics with GCP underwent simplified papilla preservation flap surgery under conventional (Control 30 quadrants) and microsurgical approach (Test 30 quadrants). Early Wound Healing Index (EWHI) was evaluated at first and second week post- intervention. GCF was collected at the time of surgery, 2nd and 6th week post -surgery for estimation of TGF- β. **RESULTS:** TGF- β at baseline was 683.78±33.14 ng/L (control) and 694.33±30.06 ng/L (Test). Post-surgery at second week, it increased in both the groups, 1048.47± 133.22 ng/L(test) and 872.51± 155.58 ng/L(control). At sixth week, the sites treated under loupes showed an increased expression (698.50± 48.35 ng/L) with statistical significance when compared to control sites (P<0.001). 100% of the test sites achieved complete flap closure at 2nd week, whereas it was 9.7% with incomplete flap closure in control sites. **CONCLUSION:** Minimally invasive periodontal flap under magnification loupes demonstrated an increased expression of TGF-β and better EWHI scores in diabetics with chronic periodontitis.

Keywords
Diabetes Mellitus; Microsurgery; Periodontal flap; Wound healing; TGF-β.

Introduction

Diabetes Mellitus and Periodontitis are chronic inflammatory diseases with an established two-way relationship [1]. The upregulated cytokine levels in periodontitis induce insulin resistance with ensuing hyperglycaemic state. Furthermore, the heightened inflammation results in greater tissue damage in diabetics [2,3]. Leukocytic and macrophage dysfunction, decreased expression of growth factors, reduced cellular proliferation, reduced extracellular matrix synthesis, and increased production of proteolytic enzymes compromise wound healing dynamics in diabetics. The enhanced apoptosis of cells like the fibroblasts further results in reduced turnover of new connective tissue matrix with enhanced tissue destruction.[4,5]

Conventional periodontal surgical techniques rely on wider incisions and access for proper visualization, however with the advent of microsurgical techniques, minimal access with enhanced visual acuity facilitates atraumatic manipulation of soft and hard tissues, thorough defect debridement with consequent faster, uneventful healing with superior aesthetic outcomes.[6]Transforming growth factor- β (TGF-β) is present during the initial phases of wound healing in three isoforms i.e TGF-β1, TGF-β2 and TGF-β.[7,8] It functions both as a stimulator and inhibitor of cellular replication, controls the production of extracellular matrix, inhibits epithelial proliferation, induces apoptosis and controls morphogenesis by mediating remodelling of extracellular matrix. Thus, it plays a crucial role in wound healing.[9,10] Reduced TGF-β expression has been observed in patients with impaired wound healing particularly in diabetics. [11] Hence TGF- β could be a valuable prognostic marker for wound healing activity following flap surgery.

Limited scientific evidence is available on TGF-β expression following Simplified Papilla Preservation Flap under loupes magnification in type II diabetic individuals with generalised chronic periodontitis. Hence, we hypothesise that the use of precise incisions with minimal flap elevation under loupes magnification (2.5x) could achieve primary wound closure and facilitate early wound healing.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4286 - 4294
Received 25 April 2021; Accepted 08 May 2021.

The present study aimed to compare and evaluate the effect of surgical flaps elevated using conventional (Macrosurgery) and microsurgical technique on the expression of TGF-β levels in Gingival Crevicular Fluid (GCF) during early wound healing in type II diabetics with Generalized Chronic Periodontitis (GCP).

Materials and methods

Ethical clearance:

Institutional Scientific and Ethical Review Board granted the ethical clearance (IRB Approval No-SRMDC/IRB/2015/MDS/No.506).Surgical procedures including the possible risk, benefits and complications were explained and both verbal and written informed consent was obtained.

Subject recruitment & allotment:

Thirty nine diabetic subjects with mild to moderate GCP were recruited and underwent non surgicalperiodontal therapy (NSPT); six weeks later thirty five subjects reported for review, four subjects were lost to follow up. Clinical parameters were re-evaluated, full mouth radiographs were taken using paralleling angle technique and periodontal flap surgery was planned for the residual pockets of PPD ≥5.4mm. Split mouth study design was planned and the quadrants were assigned to simplified papilla preservation technique (SPPF by Cortellini et al, 1999 12 by either conventional macrosurgical (Control) or microsurgical approach using 2.5x magnification loupes (Test). The treatment technique and the quadrant to be treated were decided on the day of surgery by randomisation using lottery in a sealed envelope. All the surgical procedures were performed by a single experienced periodontist (PSG).

Subjects aged between 30 to 60 years with a minimum of 20 teeth present (five in each quadrant minimum) and diagnosed with GCP based on the American Academy of Periodontology, 1999 classification 13, with Probing Pocket Depth (PPD) ≥ 5 mm and Clinical Attachment Level(CAL) ≥ 3 mm in more than 30% of sites with horizontal bone loss as evidenced by radiographs and involving a minimum of 3 teeth per quadrant in the contralateral quadrants were included. The recruited subjects were diagnosed with type II DM by a diabetologist and were under treatment with oral hypoglycemic agents for a minimium of 1 year or more with glycated haemoglobin levels (HbA1C) <8%.

Subjects with any other systemic disease that could influence the course of periodontal disease, or had taken antibiotics in the past 6 months, non compliant with poor oral hygiene, prior history of periodontal surgical intervention, radiation therapy to head and neck region, immunodeficiency disorders, poorly controlled diabetes with HbA1C >8% and /or any history of diabetic complications were excluded.

Periodontal examination:

Full Mouth Bleeding Scores (FMBS)14 and Full Mouth Plaque Scores (FMPS)15 were recorded. PPD and CAL were measured at six sites (Mesio - buccal, Mid-buccal, Disto-buccal, Mesio-palatal/lingual, Mid-palatal/lingual and Disto-palatal/lingual) around each tooth using a UNC-15 probe. The measurements were made at baseline and 3 months post-surgery. All the clinical parameters were assessed by a single calibrated examiner (BS), who was blinded to the surgical treatment and the intra-examiner calibration was done on five patients by recording the clinical parameters twice within 48 hours and kappa value of 0.89 was derived.

GCF sample collection:

Site specific GCF samples were collected from one test and one control sites with the deepest PPD just before the surgical procedure (baseline). Sites were isolated with cotton rolls and air dried to prevent salivary contamination. The micro-capillary pipettes [5µl micro-capillary pipettes, Sigma Aldrich] were placed at the entrance of the gingival sulcus allowing the pipettes to gently penetrate into the gingival crevice for 3-5 minutes. Samples contaminated with blood were not included. The samples were immediately placed in Eppendorf tubes, containing 100µl of Phosphate Buffer Solution (pH 7.4) and stored at -80◦C, until analysis.

Surgical therapy:

Patients were instructed to rinse for 30 seconds with 0.12% Chlorhexidine gluconate solution. After isolation, the surgical field was anaesthetized using 2% lignocaine with 1:80,000 adrenaline. In the control group, an oblique incision was made across the papilla from the gingival margin at the buccal line angle of the involved tooth using a no.15 blade to reach the mid interproximal portion of the papilla under the contact point of the adjacent tooth. The oblique incision was continued into the gingival sulcus in the buccal aspect of the adjacent teeth and extended to partially dissect the papilla of adjacent interdental spaces allowing elevation of buccal flap. Intrasulcular incisions were continued in the

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4286 - 4294
Received 25 April 2021; Accepted 08 May 2021.

palatal aspect of the two adjacent teeth and extended into the interdental papilla of adjacent interdental spaces following which a full thickness palatal flap including the interdental papilla was elevated. Thorough debridement was done using gracey curettes and irrigated with saline. Vertical internal mattress suturing was done using resorbable 4-0 Vicryl sutures. The entire surgical procedure was performed under normal vision without any magnification.

For the test group, similar flap design was followed using no.15 C blade and Tunneling Knives No.1 and 2 and simple loupe with 2.5x magnification. Vertical internal mattress sutures were placed using resorbable 5-0 vicryl sutures using Castroviejo needle holders and scissors. Post-operative oral hygiene instructions were given and analgesic was prescribed for all the patients. `

Figure 1 A: Simplified papilla preservation-Macrosurgical approach: Buccal view, B: Simplified papilla preservation-Macrosurgical approach: Palatal view, C: Internal vertical mattress sutures placed, D:Post-operative reduction in Probing Pocket Depth

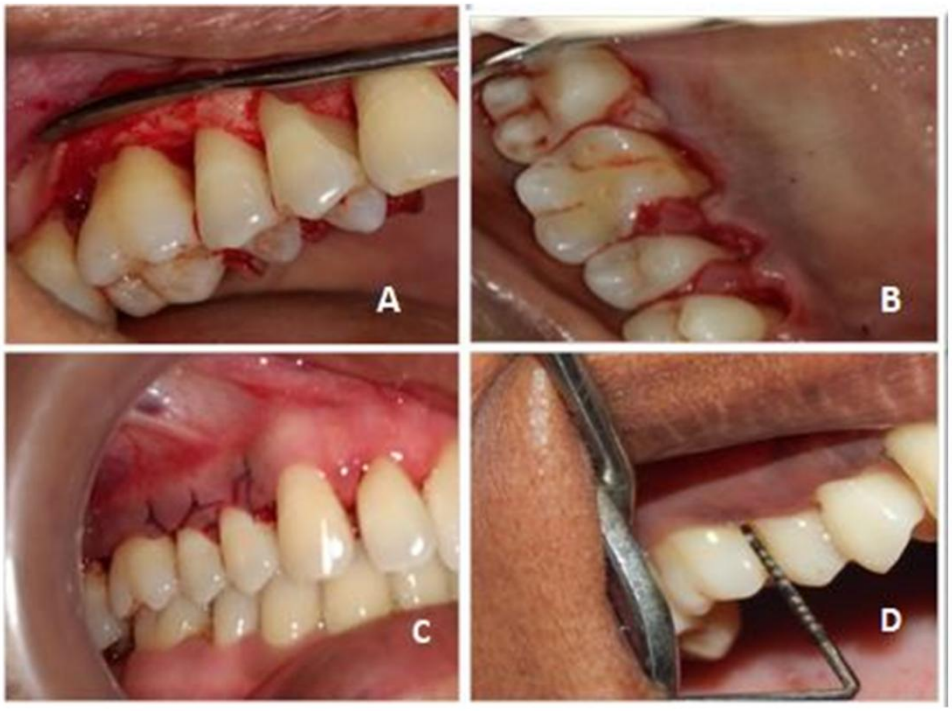
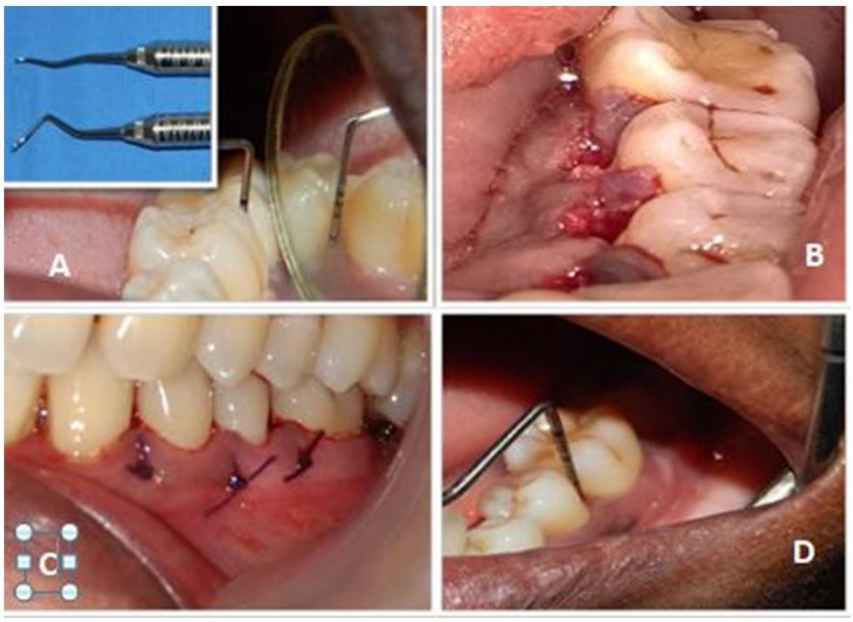


Figure 2 A:Pre-operative Probing Pocket Depth:Insert-Tunneling Knives, B: Simplified papilla preservation-Microsurgical approach: Palatal view, C: Internal vertical mattress sutures placed, D: Post-operative reduction in Probing Pocket Depth

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4286 - 4294
Received 25 April 2021; Accepted 08 May 2021.



Recall visit:

All the patients were reviewed at first week following surgery. The periodontal dressing was removed and irrigated with saline. Early wound Healing Index (EWHI) scores were recorded. The patients were re-called for suture removal on the 14th day following which EWHI scores were re-evaluated. GCF samples were again collected at 2nd and 6th week from the same baseline sites and oral hygiene instructions were reinforced at each visit. All Clinical parameters were re-evaluated at 3 months following the therapy.

Biochemical assay:

TGF-β levels in the GCF samples were determined using a commercially available Enzyme- Linked Immunosorbent Assay kit (ELISA, Bioassay Technology Laboratory, China) utilising double-antibody sandwich technique. GCF sample was added to the wells pre -coated with TGF-β monoclonal antibody. Biotin-conjugated anti-human TGF-β antibody was added following incubation and that binds to human TGF- β. Consequently, the unbound biotin-conjugated anti-human TGF- β antibody was washed away. Next, streptavidin-HRP was added and incubated, this bound to the biotin conjugated anti -human TGF- β antibody, again during the washing step unbound streptavidin-HRP was washed away. Substrate solution was added and the intensity of colour measured would be based on the concentration of TGF- β. Finally, addition of acidic stop solution terminated the reaction and the absorbance was measured at 450 nm. TGF-β levels were calculated based on the instructions given by the manufacturer.

Statistical analysis

IBM SPSS Statistics for Windows, version 22.0. Armonk, NY: IBM Corp (IBM Corp. Released 2013) was used for statistical analysis. Normality test revealed that the data followed normal distribution; hence parametric tests were applied. Intra and intergroup comparison between time points were done using paired t test and independent t test for clinical parameters. For intragroup and intergroup comparison of EWHI, Mc Nemar's Chi-square test was applied. To compare mean TGF- β values between time points and between test and control groups, paired t test and independent t test were applied respectively. *P value ≤ 0.05* was considered to be statistically significant.

Results

Table 1, shows the intra and intergroup comparison of clinical parameters. Plaque and bleeding scores reduced from baseline to three months in both the control and test group with no statistical significance(P>0.05).The control and test groups demonstrated a significant reduction in the PPD from baseline to three months i.e3.01±0.34mm and 3.13±

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4286 - 4294
Received 25 April 2021; Accepted 08 May 2021.

0.34mm respectively (P<0.001). CAL revealed a statistically significant gain in both the control and test group i.e 3.03±0.33 mm and 2.74±0.37 mm respectively from baseline to three months (P<0.001). On intergroup comparison, PPD and CAL gain showed no statistical significance (Table 1).

Table 1: Intragroup And Intergroup Comparison Of Clinical Parameters At Baseline And At 3 Months.

CLINICAL PARAMETERS	TIME INTERVAL	CONTROL (mean ±SD)	TEST (mean ±SD)	P VALUE
Full Mouth Plaque Score	Baseline	0.12±0.04	0.12±0.04	1.000[‡]
	3 months	0.10±0.06	0.10±0.06	1.000[‡]
	p Value	0.101[†]	0.101[†]	
Full Mouth Bleeding Score	Baseline	0.13±0.01	0.13±0.01	1.000[‡]
	3 months	0.11±0.02	0.11±0.02	1.000[‡]
	p Value	0.101[†]	0.101[†]	
Probing Pocket Depth (in mm)	Baseline	6.25±0.81	6.48±0.81	0.393[‡]
	3 months	3.24±0.47	3.35±0.44	0.087[‡]
	p Value	<0.001[**†]	<0.001[**†]	
Clinical Attachment Level (in mm)	Baseline	3.51±1.02	3.43±1.47	0.073[‡]
	3 months	0.48±0.69	0.69±1.10	0.466[‡]
	p Value	<0.001[**†]	<0.001[**†]	

[‡]INDEPENDENT T TEST, [†]PAIRED T TEST
[**]P<0.001 HIGHLY SIGNIFICANT
SD:STANDARD DEVIATION

Table 2, shows overall EWHI scores of study group at first and second week post-surgery.In the control group complete flap closure was achieved in 58.3% and 90.3% of sites at first week, and 2nd week indicating that even at 2 weeks post-surgery9.7% of sites did not achieved complete flap closure. On the contrary, in the test group at the first week 80.3% at second week 100% sites were completely healed..The difference in the healing scores between the time points within the groups was statistically significant (P≤0.001). (Table 2)

Table 2: Intragroup And Intergroup Comparision Of Overall EWHI Scores At Different Time Points

Parameter	Time points	Control group		Test group		P value
		N	%	N	%	

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4286 - 4294
Received 25 April 2021; Accepted 08 May 2021.

EWHI-score 1	1st week	42	58.3	57	80.3	0.004**‡
	2nd week	65	90.3	71	100	0.013*‡
p value		<0.001**‡		<0.001**‡		
EWHI-score 2	1st week	30	41.7	14	19.7	0.004**‡
	2nd week	7	9.7	0	.0	0.013*‡
p value		<0.001**‡		<0.001**‡		

**P<0.001 HIGHLY SIGNIFICANT, *P<0.05 SIGNIFICANT
‡Mc-NEMAR CHI-SQUARE TEST

Table 3, shows the intra-group comparison of TGF-β levels in the control and test group at three different time points.In both the study groups, the TGF-β levels increased at second week post-surgery (P≤0.001) and consequently, at six weeks the levels significantly reduced below the baseline values (P≤0.001) in control group and remained similar to the baseline value in the test group.

Table 3: Intragroup Comparision Of TGF-β In Control And Test Groups

Time points	Control group (mean ±SD)	P value	Test group (mean ±SD)	P value
TGF- β (Baseline)	683.78±33.14	<0.001**†	694.33±30.66	<0.001**†
TGF- β (2 weeks)	872.51±155.58		1048.47±133.22	
TGF- β (Baseline)	683.78±33.14	<0.001**†	694.33±30.66	0.705
TGF- β (6 weeks)	579.19±73.31		698.50±48.35	
TGF- β (2 weeks)	872.51±155.58	<0.001**†	1048.47±133.22	<0.001**†
TGF- β (6 weeks)	579.19±73.31		698.50±48.35	

**P<0.001 HIGHLY SIGNIFICANT
†PAIRED T TEST
SD:STANDARD DEVIATION

Table 4, shows the intergroup comparison of TGF-β between the control and test group at three different time points. On comparing the TGF-β levels between the control and the test group at different time points, it was observed that baseline levels were similar, but at 2 weeks post-surgery the test group had a twofold increase in TGF-β expression when compared to the control group. When comparing the TGF-β levels at 6 weeks, both the groups had a reduction in TGF-β levels from 2 weeks but the test group demonstrated increased expression even at 6 weeks in comparison with the control group.

Table 4: Intergroup Comparision Of TGF-β Between Control Control And Test Group

VARIABLES	MEAN ±SD	P-VALUE
TGF- β (Baseline): CONTROL	683.78±33.14	0.207‡
TGF- β (Baseline) : TEST	694.33±30.06	
TGF- β (2 weeks) : CONTROL	872.51±155.58	<0.001**‡
TGF- β (2 weeks): TEST	1048.47±133.22	
TGF- β (6 weeks) : CONTROL	579.19±73.31	<0.001**‡
TGF- β (6 weeks): TEST	698.50±48.35	

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4286 - 4294
Received 25 April 2021; Accepted 08 May 2021.

**P<0.001 HIGHLY SIGNIFICANT
‡INDEPENDENT T TEST
SD:STANDARD DEVIATION

Table 5, shows the site specific intra and inter-group comparison of mean TGF-β and EWHI scores at second week. EWHI scores at 2nd week in the control group, revealed that 25 out of the 30 sites, had a complete flap closure (Score 1) and when the healing was compared with the TGF- β expression, the levels remained almost similar with no statistical significance (P=0.834). All the sites in the Test group showed complete flap closure (Score 1) at 2nd week and elevated TGF- β levels than the control group. The data revealed that test group had complete flap closure along with elevated TGF-β levels when compared with the control group at 2 weeks.

Table 5: Site Specific Comparision Of TGF-β And EWHI Scores At 2 Weeks:

GROUPS	EWHI (2nd WEEK)		TGF-β (2nd WEEK)		P-VALUE
	SCORE	N	MEAN	SD	
CONTROL	1	25	869.78	170.62	≤0.834‡
	2	5	886.15	23.17	
TEST	1	30	1048.47	133.22	-
	2	0	-	-	

‡Mc-NEMAR CHI-SQUARE TEST
SD:STANDARD DEVIATION

Discussions

Diabetic individuals are highly susceptible to periodontal disease and appropriate periodontal management can reduce diabetic complications and improve glycemic status.16The conventional open flap debridement procedures involve extensive flap reflection leading to tissue trauma, postoperative inter- dental papillary loss, discomfort and pain. In diabetic individuals, these problems are further exaggerated as the periodontium is already primed to respond with multiple levels of host compromise. Therefore, clinicians need to understand the complex dynamics that cause disease, and be able to appropriately deliver therapeutic interventions that are able to overcome this inherent systemic compromise. In this context, application of minimally invasive atraumatic surgical techniques facilitates faster healing and regeneration of the periodontal tissues due to passive wound closure and primary intention healing.6Till date no clinical studies have compared microsurgical and conventional periodontal flap surgery in chronic periodontitis patients with Type II Diabetes Mellitus.

The plaque and bleeding scores as well as the gain in clinical attachment and probing pocket depth reduction of the control and test groups were similar indicating that both the groups benefitted evenly from the continual monitoring and motivation. The design of this minimally invasive surgical approach enhanced wound stability during the early wound healing period. Thus the improved stability of soft tissues could play a positive role in increasing the stability of the blood clot, a key factor in periodontal regeneration.17 The results were in agreement with Meenapriya et al.18 The significant difference in CAL gain in both the groups from baseline highlights the fact that diabetics also respond to periodontal surgical procedure in a similar manner to that of non diabetic subjects which was in agreement with the study done by Sheetal Oswal et al.19

EWHI scores revealed that all the test sites healed completely at 2 weeks. This was in agreement with the study done by Wachtel et al20 and Cortellini et al21 The strong point of the current study is that, minimal magnification achieved better healing in diabetics underscoring the importance of precise incisions, minimal flap reflection and approximation in the microsurgery group, which favoured faster wound healing despitethe compromised host response.

TGF-β was detected in all the GCF samples from deepest pocket sites and this was in accordance with Sattariet al 11 and Steinsvollet al 23. These authors suggested that up regulation of TGF-β possibly occurs to compensate for the

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4286 - 4294
Received 25 April 2021; Accepted 08 May 2021.

destructive host inflammatory responses in periodontitis patients.Studies have shown that and the levels of TGF- β in gingival tissues may be valuable in detecting inflammatory reaction of periodontal tissues.24,25

TGF-β levels at different time points of the present study indicate that the microsurgical group had a greater release of growth factors during the initial phases of healing indicating that the application of minimally invasive techniques in diabetic wound sites will demonstrate better and faster wound healing. Our findings were in agreement with Kuru et al 26, wherein they demonstrated that TGF-β was detected in all the GCF samples till 6 weeks but only 90% of sites had TGF-β at 12th weekpost surgery. The increase in TGF- β levels post surgery was in contrary to the findings of Vikram et al, wherein they observed decreased levels from baseline at 2 weeks post periodontal flap surgery in the GCF of chronic periodontitis patients.27GCF TGF- β concentrations decreased statistically from baseline to 6 weeks post-surgery in this study and these findings were in agreement with Kuru et al26 and Mandana Sattari et al.11 such a observation could perhaps be attributed to the reduction in bacterial toxins following elimination of microbial load followed by the reduction in the inflammatory mediators.

Conclusions

Within the study limitations, microsurgical technique under optimal magnification using 2.5x loupes, in well controlled diabetic subjects facilitates precision in flap procedure, thereby resulting in minimal tissue injury and better wound healing. This is the first study to demonstrate the expression of GCF TGF-β in diabetics with chronic periodontitis following periodontal microsurgery. Thus, minimally invasive flap management supports an undisturbed maturation of the underlying fibrin clot during the early healing phase, and may be equally important in providing a robust environment favouring the early events of wound healing. Nevertheless, a longer period of evaluation may be necessary in future clinical trials to appreciate the clinical effectiveness of this technique and to evaluate its long term benefits.

Limitations and Future Studies

Limitation of the study could be the short- term follow up period for evaluating the clinical parameters. However, the primary objective of the study was to evaluate the expression of TGF- β pre and post periodontal flap surgical management and the maximum expression was anticipated during the initial phases of wound healing, hence the follow up of 3 months seemed appropriate.

Acknowledgement –Nil
Conflict of interest: nil
Source of funding: self funded

The manuscript has not been published and is not under consideration for publication in any other journal .
All authors approved the manuscript and its submission to the journal.
The manuscript has been submitted solely to this journal and is not published, in press, or submitted elsewhere. All the research meets the ethical guidelines, including adherence to the legal requirements of the study country.

References

[1] Negrato CA, Tarzia O, Jovanovič L, Chinellato LE. Periodontal disease and diabetes mellitus. J Appl Oral Sci 2013;21:1.
[2] Emrich LJ, Shlossman M, Genco RJ. Periodontal disease in non-insulin-dependent diabetes mellitus. J Periodontol1991;62:123-31.
[3] Khader YS, Dauod AS, El-qaderi SS, Alkafajei A, Batayha WQ. Periodontal status of diabetics compared with nondiabetics: a meta-analysis. J Diabetes Complications 2006 28;20:59-68.
[4] Bascones-Martinez A, Matesanz-Perez P, Escribano-Bermejo M, González-Moles MÁ, Bascones-Ilundain J, Meurman JH. Periodontal disease and diabetes-Review of the Literature. Med Oral Patol Oral Cir Bucal 2011;16:e722-729.

[5] Chang PC, Chung MC, Wang YP, Chien LY, Lim JC, Liang K, et al. Patterns of diabetic periodontal wound repair: a study using micro-computed tomography and immunohistochemistry. J Periodontol 2012;83:644-52.
[6] Shanelec DA, Tibbetts ÔS. Periodontal microsurgery. J Esthet Restor Dent 2003;15:402-7.
[7] Lawrence WT, Diegelmann RF. Growth factors in wound healing. Clin Dermatol 1994;12:157-69.
[8] Steenfos HH. Growth factors and wound healing. Scand J Plast Reconstr Surg Hand Surg 1994;28:95-105.
[9]Tendijke P, Iwata KK. Growth factors for wound healing. Nat Biotechnol 1989;7:793-8.
[10] Isogai N, Ueda Y, Kurozumi N, Kamiishi H. Wound healing at the site of microvascular anastomosis: Fibrin-stabilizing factor XIII administration and its effects. Microsurgery 1990;11:40-6.
[11] Sattari M, Fathiyeh A, Gholami F, Tamijani HD, Samani MG. Effect of Surgical Flap on IL-1 [Beta] and TGF-[Beta] Concentrations in the Gingival Crevicular Fluid of Patients with Moderate to Severe Chronic Periodontitis. Iran J Immunol 2011 1;8:20.
[12] Cortellini P, Prato GP, Tonetti MS. The simplified papilla preservation flap. A novel surgical approach for the management of soft tissues in regenerative procedures. Int J Periodontics Restorative Dent 1999;19.
[13] Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999;4:1-6.
[14]Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J 1975;25: 229–235.
[15] O'leary TJ, Drake RB, Naylor JE .The plaque control record. J Clin Periodontol 1972; 43: 38.
[16] Taylor GW, Burt BA, Becker MP, Genco RJ, Shlossman M, Knowler WC, Pettitt DJ. Non-insulin dependent diabetes mellitus and alveolar bone loss progression over 2 years. J Periodontol 1998;69:76-83.
[17]Wikesjö UM, Selvig KA. Periodontal wound healing and regeneration. Periodontol 2000 1990;19:21-39.
[18] Perumal MP, Ramegowda AD, Lingaraju AJ, Raja JJ. Comparison of microsurgical and conventional open flap debridement: A randomized controlled trial. J Indian Soc Periodontol 2015;19:406.
[19]Oswal SK, Dwarkanath CD, Ramesh AV. Evaluation of periodontal surgical procedures in type-2 diabetic patients. Int J Stomatol occlusion Med 201;4:59.
[20]Wachtel H, Schenk G, Böhm S, Weng D, Zuhr O, Hürzeler MB. Microsurgical access flap and enamel matrix derivative for the treatment of periodontal intrabony defects: a controlled clinical study. J Clin Periodontol 2003;30:496-504.
[21]Cortellini P, Tonetti MS. Microsurgical approach to periodontal regeneration. Initial evaluation in a case cohort. J Periodontol 2001;72:559-569.
[22]Steinsvoll S, Halstensen TS, Schenck K. Extensive expression of TGF-b1 in chronically-inflamed periodontal tissue. J Clin Periodontol 1999;26:366-373.
[23]Skalerič U, Kramar B, Petelin M, Pavllia Z, Wahl SM. Changes in TGF-β1 levels in gingiva, crevicular fluid and serum associated with periodontal inflammation in humans and dogs. Eur J Oral Sci 1997;105:136-42.
[24]Ko WL, Wang JC, Chen CC, Wu YM, Tsai CC. TGF-beta 1 in the experimentally induced inflammatory periodontal tissues in miniature swines. Kaohsiung J Med Sci 1999;15:315-21.
[25]Gürkan A, Emingil G, Çinarcik S, Berdeli A. Gingival crevicular fluid transforming growth factor-β 1 in several forms of periodontal disease. Arch Oral Biol 2006;51:906-912.
[26]Kuru L, Griffiths GS, Petrie A, Olsen I. Changes in transforming growth factor-β1 in gingival crevicular fluid following periodontal surgery. J Clin Periodontol 2004;31:527-33.
[27]Vikram V, Ramakrishnan T, Anilkumar K, Ambalavanan N. Changes in transforming growth factor-β1 in gingival crevicular fluid of patients with chronic periodontitis following periodontal flap surgery. Journal of clinical and diagnostic research: J Clin Diagn Res. 2015;9:ZC13.