

# 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

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## ABSTRACT

**Background:** Growth factors play a major part in wound healing in many tissues including the periodontium. Transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) is one of these factors present in the gingival crevicular fluid. In addition, it is considered as one of the most important anti-inflammatory cytokines. Interleukin-1 $\beta$  is a proinflammatory cytokine that presents itself in gingival inflammation and the GCF. Such factors might be of value as prognostic markers of wound healing activity and the therapeutic progress following flap surgery. **Objective:** The aim of this study was to evaluate the effect of surgical flap on the concentration of IL-1 $\beta$  and TGF- $\beta$  in the GCF of patients with moderate to severe chronic periodontitis. **Methods:** The GCF samples were collected, using the Perio-Paper strip at phase 1 (pre-surgery), phase 2 (4th week post surgery) and phase 3 (12th week post surgery) from 20 sites of 10 patients undergoing flap surgery. After the elution, IL-1 $\beta$  and TGF- $\beta$  concentrations were measured by enzyme-linked immunosorbent assay (ELISA). **Results:** The mean TGF- $\beta$  and IL-1 $\beta$  concentration decreased from phase 1 to phase 3 ( $p < 0.05$ ). There were no significant statistical correlations between IL-1 $\beta$  and TGF- $\beta$ 1 concentrations in the 3 assessment phases. There was a significant statistical correlation between TGF- $\beta$ 1 concentrations and the Plaque Index (PI) in phase 2 ( $p < 0.05$ ). There was a significant statistical correlation ( $p < 0.05$ ) between IL-1 $\beta$  and TGF- $\beta$ 1 concentration and the probing pocket depth (PPD). **Conclusion:** The flap surgery has a significant effect on decreasing IL-1 $\beta$  concentration. In the case of TGF- $\beta$ 1, probably the decrease in the concentration after treatment might be due to the removal of the inflammatory stimulants.

**Key words:** Periodontitis, Periodontal Surgical Therapy, Cytokine, IL-1 $\beta$ , TGF- $\beta$

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## INTRODUCTION

Although periodontal bacteria are the cause of periodontitis, subsequent progression and the severity of the disease are thought to be determined by the host immune responses (1). Like endodontopathic bacteria, periodontopathic bacteria stimulate cells comprising periodontal tissues to express various inflammatory cytokines such as IL-1 $\beta$  (2,3). These cytokines may activate the production of matrix metalloproteinases (MMPs) and prostaglandin E2 (PGE2) and the differentiation of osteoclasts and result in connective tissue destruction and alveolar bone resorption (4). IL-1 $\beta$  is a glycoprotein of 17 KDa and was originally identified as an endogenous pyrogen and has been well characterized as a major proinflammatory cytokine that plays pleiotropic roles in host defense by inducing acute and chronic inflammation through activation of the innate and acquired immune systems (5,6). It is produced by activation of multiprotein complexes, termed inflammasomes, and assembles in the cytoplasm of the cells (7).

In periodontitis patients, increased IL-1 $\beta$  levels have been reported in both GCF (8,9) and periodontal tissues (10,11). The amounts of IL-1 $\beta$  have been closely associated with periodontal disease severity (12). However, tissue destruction could be protected by suppressing the activity of proinflammatory cytokines by anti-inflammatory cytokines such as TGF- $\beta$ 1, which is predominantly produced by T regulatory (Treg) cells and macrophages (13) and could also induce a wide range of essential functions including activation, proliferation, migration and the synthesis of extracellular matrix (ECM) components (14,15). Thus it is likely that these factors may also play a major part in mediating the re-building of healthy periodontal ligament (PDL) (16,17). TGF- $\beta$ 1 is present during initial phases of wound healing (18). It has highly diverse biological effects including the chemotactic and the mitogenic activity of gingival and periodontal ligament fibroblasts (19-23), and the upregulation of ECM components including collagen, fibronectin, tenascin and proteoglycans (24,25). Three isoforms, TGF- $\beta$ 1, TGF- $\beta$ 2 and TGF- $\beta$ 3 have been described (17). It has also been found that TGF- $\beta$  has some degree of clinical efficacy in promoting periodontal regeneration (26,27). The precise role of TGF- $\beta$  in periodontal wound healing remains unclear.

Thus, it is postulated that the balance between inflammatory mediators and their counter-regulatory cytokines has an important role in determining the outcome of the lesion, especially after treatment. Therefore, the aim of this study was to compare the effects of periodontal conventional flap (CF) on the levels of IL-1 $\beta$  and TGF- $\beta$ 1 in GCF samples of patients with moderate to severe periodontitis.

## MATERIALS AND METHODS

**Subjects.** GCF samples were obtained from a total of 55 interproximal pockets in 11 male and 17 female patients with a mean age of  $35.1 \pm 6.8$  yrs, who were suffering from moderate to severe chronic periodontitis. They were treated with scaling and root planning and oral hygiene instructions before periodontal surgery and there was radiographic evidence of bone loss after completion of the initial periodontal therapy (Scaling and root planning) and before surgical treatment. Patients were excluded from the study for the following conditions:

- Systemic illness
- Chronic medication known to affect the periodontal status within 4 weeks of the baseline examination
- Antibiotic therapy within 3 months of baseline examination
- Periodontal therapy within 6 months of the baseline examination
- Pregnant or lactating females
- Smoking
- Oral disease other than periodontal disease

Verbal informed consents were obtained from all recruits. The protocol was approved by the Dental Research Center and Ethics Committee of the Dental School of Shahid Beheshti University of Medical Sciences.

**Flap surgery.** A total of 55 interproximal defects were treated by conventional flap (CF) surgery. All the flaps were performed by one therapist. Following reverse bevel incisions, mucoperiosteal flaps were raised, granulation tissues removed and root instrumentation performed. The flaps were replaced to their original position and secured with sutures and covered with periopacks. Subjects were advised to rinse with 0.2% chlorhexidine twice daily for a period of two weeks. All the operative procedures were performed under local anesthesia with 2% Xylocaine. The sutures were removed one week after surgery and the subjects were recalled 4 and 12 weeks after the initial surgery for collecting GCF samples. They were also recalled at 4-week intervals after surgery for professional tooth cleaning up to 6 months after the initial surgery. Although the primary objective of this study was not the measurements of probing pocket depth (PPD), clinical attachment loss (CAL) and plaque index (PI), nevertheless PPD and CAL were carried out before and 12 weeks after the surgery by Williams periodontal probe (Hu Friedy™, Chicago IL, USA) at 6 sites per tooth. Clinical measurements were performed immediately after GCF collection. However, PI was assessed in all three phases of the study (before, 4 and 12 weeks after surgery). The PI was recorded dichotomously during GCF collection. GCF samples were collected immediately before surgery and at 4 and 12 weeks post-surgery. The selected sites were isolated with cotton roles, saliva removed using a fine-bore high-power suction tip. Periopapers (Oraflow, Smithtown, NY, USA) were placed at the entrance of the crevice and left in position for 2 minutes. The volumes of collected GCF were measured before and after sample collection using a Sartorius microbalance accurate to within 0.001 mg. Then they were transferred in to separate microcentrifuge tubes and were stored at -80 °C prior to use. Periopapers with any visible sign of saliva or blood contamination were discarded.

**ELISA.** The GCF blotted periopapers were allowed to thaw at room temperature for 30 minutes. For elution of the GCF samples, 250 microliter of phosphate-buffered saline (PBS) was added to each microcentrifuge tube containing one periopaper and centrifuged at 2.0124×g for 30 minutes. Following elution, each GCF sample was analyzed separately. Then, ELISA method was used in order to measure the concentration of TGF-β1 (BMS249/2 Human TGF-β1 ELISA Kit, Bender MedSystems, Austria) and IL-1β (BMS224/2 Human IL-1β ELISA Kit, Bender MedSystems, Austria). The operator and the person responsible for recording the results had no prior knowledge of the group to which the samples belonged.

**Statistics.** Since in this study Kolmogorov-Smirnov test showed that the distribution of data was not normal so statistical analysis was made by the aid of non-parametric tests including; Mann-Whitney U, Chi-Square and Spearman's correlation coefficient tests. A p value of  $\leq 0.05$  was considered statistically significant.

## RESULTS

We found IL-1 $\beta$  and TGF- $\beta$ 1 in all of the 55 samples in three different phases. The mean levels of IL-1 $\beta$  and TGF- $\beta$ 1 (ng/ml) in GCF samples at baseline (phase 1), 4 weeks (phase 2) and 12 weeks after surgery (phase 3) are summarized in Table 1.

**Table 1. Mean levels  $\pm$  standard deviation (SD) of IL-1 $\beta$  and TGF- $\beta$ 1 (ng/ml) in GCF samples at baseline, one month and 3 months after surgery.**

	IL-1 $\beta$	TGF- $\beta$ 1
Baseline (Phase 1)	125.80 $\pm$ 78.27	0.65 $\pm$ 0.7
1 month after surgery (Phase 2)	68.63 $\pm$ 69.66	0.42 $\pm$ 0.18
3 months after surgery (Phase 3)	53.25 $\pm$ 57.07	0.36 $\pm$ 0.08

The mean concentration of IL-1 $\beta$  in GCF samples in phase 1 to 3 was 125.8  $\pm$  78.27, 68.63  $\pm$  69.66 and 53.25  $\pm$  57.07 (ng/ml), respectively. The mean concentration of TGF- $\beta$ 1 in GCF samples in phase 1 to 3 were 0.65  $\pm$  0.7; 0.42  $\pm$  0.18 and 0.36  $\pm$  0.08 (ng/ml), respectively.

Chi-square test showed a significant decrease in the mean level of IL-1 $\beta$  from baseline (phase 1) to 3 months after CF or phase 3 ( $p < 0.01$ ) by using Mann-Whitney U test. There were significant difference ( $p < 0.05$ ) between phases 1 and 2 and also between phase 1 and 3 ( $p < 0.01$ ) but the difference between phase 2 and 3 was not significant. There was also a significant decrease in TGF- $\beta$ 1 level from phase 1 to phase 3 ( $p < 0.01$ ). Like IL-1 $\beta$ , we also found significant differences between phase 1 and 2, and phase 1 and 3 regarding TGF- $\beta$ 1 levels using Mann Whitney U test ( $p < 0.01$ ). In other words, the maximum levels of the IL-1 $\beta$  or TGF- $\beta$ 1 have been found in the baseline and the minimum levels in phase 3.

Spearman correlation coefficient test showed no significant correlation between IL-1 $\beta$  and TGF- $\beta$ 1 in any of the above mentioned phases, but there was a significant statistical negative correlation between the level of TGF- $\beta$ 1 and plaque index (PI) in phase 2 ( $p < 0.05$ ). There were also significant correlations between the level of IL-1 $\beta$  and probing pocket depth (PPD), and IL-1 $\beta$  level and PI in phase 3 ( $p < 0.05$ ).

## DISCUSSION

We found TGF- $\beta$ 1 in all of GCF samples and there was a significant decrease in TGF- $\beta$ 1 level from phase 1 (baseline or before surgery) to phase 3 (12 weeks after surgery).

Our findings are in agreement with those of Kuru et al. where TGF- $\beta$ 1 has been found in all and 90% of GCF samples at 4th week and 12th week after surgery, respectively (28). They showed that TGF- $\beta$ 1 remained high at these sites until 4 weeks post-surgery but thereafter declined to levels that were lower than baseline values. This is also consistent with previous observations that during healing after periodontal treatment, newly synthesized connective tissue components including collagen types I and III and fibronectin are present at elevated levels in the GCF of treated sites (29-31). Periodontal therapy has also been shown to modulate GCF levels of glycosaminoglycans (32), matrix metalloproteinases (33,34) and their tissue inhibitors (34,35).

Four weeks after CF, we also found a significant statistically negative correlation between the level of TGF- $\beta$ 1 and plaque index (PI). In this regard, Skaleric et al. reported higher GCF TGF- $\beta$  levels in sites with deeper periodontal pockets than the less involved sites in periodontal patients (36).

We also detected IL-1 $\beta$  in all of GCF samples and similar to TGF- $\beta$ 1; there was a significant decrease in its concentration from phase 1 to phase 3. This interpretation is consistent with the findings of Zhang et al. who reported that IL-1 and TNF have a detrimental effect in the early phase of periodontal wound healing (37). Decrease in IL-1 $\beta$  following CF is also in agreement with the findings of several studies which reported that following periodontal therapy, there was a reduction in the levels of IL-1 $\beta$  in GCF (38-42). Goutoudi et al. found that at 6 weeks following periodontal therapy, the total amounts of IL-1 $\beta$  were found to be significantly reduced in all sites (43).

12 weeks after CF, we found a positive correlation between PI and IL-1 $\beta$  and between PPD and the level of IL-1 $\beta$ . This is in agreement with several previous studies (40,41,44). Gonzáles et al. demonstrated no correlation between the amounts of IL-1 $\beta$  and clinical parameters in gingivitis (45). However, in several studies it was shown that overproduction of IL-1 significantly induces periodontal tissue destruction and inflammation (46,47).

Therefore, we conclude that removal of bacterial plaque reduces antigenic stimuli and consequently could modulate the proinflammatory cytokines and growth factors which are present in GCF. The dynamic interaction between different kinds of cytokines may help monitor the effectiveness of periodontal treatment as a diagnostic marker. Certainly GCF cytokine profiles vary with respect to the time of sampling. In the case of TGF- $\beta$ 1, it is concluded that it would decrease in concentration with time, due to the removal of inflammatory stimulants. Since we did not assess the changes in TGF- $\beta$ 1 concentration between phase 1 and phase 2, additional studies should be undertaken to elucidate further the role of TGF- $\beta$ 1 on wound healing mechanisms in more phases after periodontal surgery.

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