CLINICAL RESEARCH

The Effect of Gingival Retraction Procedures on Periodontal Indices and Crevicular Fluid Cytokine Levels: A Pilot Study

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Purpose: **The purpose of this study was to examine the effects of placement of retraction cord subgingivally upon periodontal indices including plaque index (PI), gingival index (GI), pocket depth (PD), bleeding on probing (BOP), and attachment level (AL), as well as gingival crevicular fluid (GCF) and TNF-α levels.**

Methods: **Ten teeth in 6 patients who were periodontally healthy were selected. These teeth had pocket depths of 3 mm or less, no evidence of significant loss of attachment, BOP, or plaque accumulation. The patients each received an oral prophylaxis. The following week, baseline measurements of periodontal indices and TNF-α were taken and the retraction cord was placed for 15 minutes. Following removal, the patients were dismissed. The periodontal indices measured included PI, GI, PD, BOP, and AL. In addition, the levels of TNF-α in GCF, were investigated. These measurements were made before gingival retraction as a baseline and on the 1st, 3rd, 7th, 14th, and 28th days post retraction.**

Results: **A repeated measures ANOVA showed that TNF-α levels in GCF were significantly increased at all five intervals after gingival retraction compared to the baseline. The mean TNF-α level peaked at Day 1 (0.90 ± 0.62), then declined at Days 3 (0.53 ± 0.16), 7 (0.43 ± 0.08), 14 (0.47 ± 0.10), and 28 (0.43 ± 0.08) but was still elevated 54% above baseline at Day 28, $p < 0.01$. The GI was significantly elevated at Day 1 (0.9 ± 0.49), $p < 0.01$; Day 3 (0.53 ± 0.32); and Day 7 (0.33 ± 0.33), $p < 0.05$. Unlike TNF-α, GI recovered to the baseline by day 14. Other periodontal parameters, PI, PD, BOP, and AL were not significantly altered by the gingival retraction procedure.**

Conclusion: **This pilot study supports the previous research that gingival retraction causes an acute injury that heals clinically in 2 weeks as is indicated by the GI. It also provides the first evidence that gingival retraction results in an elevation of the proinflammatory cytokine, TNF-α, in GCF.**

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GINGIVAL RETRACTION is an important aspect of current impression technique in fixed prosthodontic procedures.[1,2] Small-diameter cords packed into the gingival sulcus serve to dilate the soft tissue and expose the finish line for an accurate impression of the abutment teeth.[3] Compression of the cord into the sulcus displaces the gingival tissue and may break the gingival fiber system that connects the marginal gingivae and the cemental surface of the tooth.[4] Gingival recession is sometimes seen.[5-7] The injury associated with cord packing usually heals within 1 to 2 weeks clinically and histologically.[5-9] The injury from cord packing is often accompanied by swelling, pain, and discomfort. Occasionally, significant infection or loss of attachment occurs.[5,7] In some respects the changes in the epithelial attachment and connective tissue fiber system associated with cord retraction parallel those observed with periodontitis, where detachment of the gingival fiber system from the cemental surface, apical

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migration of the epithelial attachment, and alteration of the cemental surface are observed.[9] In contrast to periodontitis, however, the injury produced by placement of the cord is reversible and self-limited.[5,6,8]

Clinical diagnostic indices have been developed to identify the degree of severity of gingival and periodontal disease.[10-12] These are highly reproducible and closely related to the clinical stages of periodontal disease. Indices include the gingival index (GI), the plaque index (PI), the probing depth index (PDI), bleeding on probing (BOP), and attachment level (AL).

Inflammatory mediators, such as TNF-α, IL-1β, and PG-E2, play a critical role in the pathogenesis of periodontal disease and are used as markers in diagnosis and assessment of the level of disease activity, as well as in the efficacy of therapy.[13-16] Proinflammatory cytokines, particularly IL-1β and TNF-α, have been used to assess the host response to periodontal pathogens.[17] Several investigators have detected significant elevated TNF-α levels in gingival crevicular fluid (GCF) in patients with gingivitis and periodontitis.[16-18] Bostrom et al[19] demonstrated significant increased concentration of TNF-α in GCF in smoking-associated periodontal disease. Baqui et al[20] reported a significant increase of TNF-α in GCF from HIV-infected patients with periodontitis in comparison to HIV-free controls.

TNF-α, a proinflammatory cytokine and a 17 kDa protein, produced primarily by monocytes/macrophages, has been identified as a lethal mediator of acute and chronic infection.[17,20] It is an immunological mediator capable of killing tumor cells.[14] TNF-α, like IL-1β, can induce expression of other mediators that contribute to the inflammatory response such as prostaglandins, and lytic enzymes such as collagenase, that enhance bacterial killing.[20] TNF-α and IL-1β are functionally synergistic in bone resorption and remodeling.[17] Furthermore, TNF-α has been implicated in the progression of periodontal disease.[18]

The purpose of this study was to examine the effects of gingival retraction upon the periodontium with the use of periodontal indices including PI, GI, Pocket depth (PD), BOP, and AL, as well as GCF, and assay of the intracrevicular fluid for TNF-α at five time intervals (Days 1, 3, 7, 14, and 28) after the retraction procedure.

Materials and Methods

The protocol for the study was reviewed and approved by the Institutional Research Board of the University of Medicine and Dentistry of New Jersey. Informed consent was obtained from all participants. Six patients with ages from 31 to 65 without any significant medical problems were randomly selected. All denied smoking; excessive alcohol intake; use of medications; or systemic diseases, such as cardiovascular disorders, hypertension, diabetes, or hyperthyroidism. Oral hygiene was acceptable with no clinical signs of periodontal disease.

Ten teeth were selected from the 6 patients. They included 6 premolars and 4 anterior teeth. The teeth selected for the experiment had normal color and architecture of the gingiva, no BOP, a zero PI, and a zero GI. In 4 patients, 2 teeth were selected each while 2 patients had individual teeth selected. Periodontal pocket depths were 1 to 3 mm, mobility of these teeth was not observed, and the occlusions were acceptable. Seven days before the start of the experiment, patients received scaling and prophylaxis. Oral hygiene instruction was provided.

The experimental protocol consisted of baseline measurements of the indices and intracrevicular TNF-α around the tooth. Following this, gingival cord was packed. Measurements were made after removal of the cord 1, 3, 7, 14, and 28 days following gingival retraction. Braided cords (Ultrapak knitted retraction cord, #0, Ultradent, Inc, South Jordan, UT) were used with a sufficient length to double pack around each tooth. The cord was gently packed into the sulcus of the experimental tooth and left there for 15 minutes.[21,22] It was removed while moist. No medicaments were used with the cord.

All the measurements for the GCF sample collection and the periodontal parameters were made by one trained and calibrated examiner. GCF samples for each tooth were obtained from four selected sites: mesiobuccal, distobuccal, mesiolingual, and distolingual of the tooth. The periodontal parameters at each site were measured twice and averaged. The measurements were performed in the following order: (1) GCF collection, (2) PI, (3) GI, (4) PD, (5) BOP, and (6) AL.

GCF Sampling

The site to be sampled was isolated with cotton rolls, supragingival plaque was removed with a water rinse, if necessary, and the site was gently dried with an air syringe. A Periopaper strip (Pro Flow Inc., Amityville, NY) was inserted into the gingival sulcus until mild resistance was felt, and kept in place for 30 seconds. Samples contaminated with blood were discarded. Immediately after collection, the volume of GCF on the strip was measured with a calibrated Periotron

6000 meter (IDE Interstate, Amityville, NY). Then the paper strip was placed in an ependorph tube and frozen at 30°C for ELISA analysis. Calibration of the Periotron was accomplished prior to GCF collection with distilled water using a 2 μl pipette in increments from 0.1 to 0.9 μl. Each volume was applied three times to a paper strip and the Periotron units were recorded. Based on these readings a calibration curve was drawn. The calibration was done twice during the 4-week experiment.

Biochemical Assay

The levels of TNF-α were assayed by a high-sensitivity Quantikine ELISA kit (R&D Systems, Minneapolis, MN) with recombinant TNF-α monclonal antibody as a standard. All procedures followed manufacturer's instructions. Samples were removed from the freezer 24 hours prior to assay and eluted with 200 μl of PBS buffer (Dulbecco's phosphate buffered saline, Gibco BRL Co., Gaithersburg, MD). The strips were removed and the fluid was extracted for analyses. The fluid from each sample was placed in an individual well and incubated for 3 hours. The conjugate, substrate, amplifier, and stop solutions were added to each sample as per manufacturer's instructions. The peroxidase-substrate color reaction was read on a plate reader (EL312, Bio-Tek, Winooski, VT) at a wavelength of 490 nm. TNF-α was quantified to pg/ml using a known standard curve for optical density. This curve was established using a standard solution that was diluted 5 times. Due to pretest precision (correlation of $r = 0.994$ between the first and the second measurement) double testing of the samples was not done.[23] As noted previously, readings from the Periotron 6000 were converted into GCF volumes using the calibration curve previously plotted. The amount of TNF-α per sample was calculated by dividing the amount of TNF-α by the GCF volume in the sample in pg/ml.

Statistical Analysis

Descriptive data including means and standard deviations (SD) were obtained for all ten sample teeth at each of the experimental periods for each of the indices and the TNF-α levels. A repeated-measures ANOVA was used to test for differences between different time groups, $p < 0.05$.

Results

The effects of retraction procedures upon the periodontal indices are presented in Table 1. Specifically, significant increases of GI were seen at days 1, 3, and 7 after gingival retraction (Fig 1). PI, BOD, and AL did not differ pretreatment and post treatment.

GCF flow rate did not show significant changes over the experimental period. The ANOVA test showed that the mean TNF-α levels in GCF were significantly increased at all five postgingival retraction visits compared with baseline (Table 1, Fig 2). TNF-α reached a peak at one day immediately after retraction, then gradually declined at days 3, 7, 14, and 28. But even at day 28, TNF-α did not recover to the baseline level ($p \leq 0.01$).

Discussion

This study is one of the first to examine the effects of gingival retraction placement cord upon periodontal indices and intracrevicular cytokine levels. Similar to previous histological studies, placement of the cord resulted in a reversible elevation in the GI suggestive of an injury to the periodontium.[1,4,7,24] The injury to the gingival

Table 1. Periodontal Parameters

	Baseline	*Day 1*	*Day 3*	*Day 7*	*Day 14*	*Day 28*
GCF (μl/s)	0.54 (0.26)	0.73 (.33)*	0.67 (0.43)	0.66 (0.52)	0.58 (0.37)	0.60 (0.32)
PI	0.15 (0.09)	0.15 (0.12)	0.09 (0.09)	0.15 (0.12)	0.07 (0.12)	0.08 (0.14)
GI	0.05 (0.11)	0.9 (0.49)**	0.53 (0.32)**	0.33 (0.33)*	0.15 (0.25)	0.03 (0.08)
A level B (mm)	5.2 (1.03)	5 (0.82)	4.9 (1.20)	4.7 (1.16)	5.1 (0.88)	5.2 (0.79)
A level L (mm)	4.4 (0.84)	5.1 (0.88)	5 (1.15)	4.1 (0.74)	3.8 (0.79)	4.1 (0.74)
PD buccal (mm)	1.90 (0.27)	1.90 (0.34)	1.93 (0.34)	1.83 (0.39)	1.73 (0.44)	1.80 (0.45)
PD lingual (mm)	2.13 (0.39)	2.33 (0.16)	2.27 (0.34)	1.90 (0.57)	1.80 (0.36)	1.73 (0.49)
TNF-α (pg/ml)	0.28 (0.51)	0.90 (0.62)**	0.53 (0.16)**	0.43 (0.08)**	0.47 (0.10)**	0.43 (0.08)**

GCF, gingival crevicular fluid; PI, plaque index; GI, gingival index; A level B, attachment level, buccal surface; A level L, attachment level, lingual surface; PD, pocket depth.
Numbers in parentheses indicate SD.
*$p \leq 0.05$; **$p \leq 0.01$.

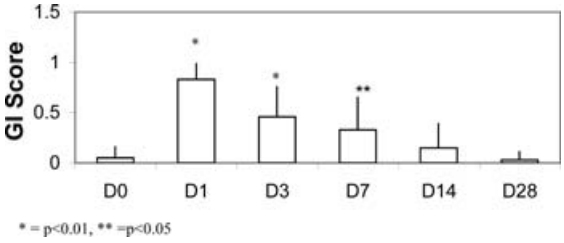

Figure 1. Mean GI at baseline and on days 1, 3, 7, 14, and 28. The GI was significantly elevated above the pretreatment level on days 1 and 3 ($p < 0.1$), and on day 7 ($p < 0.05$). Bars indicate standard deviations.

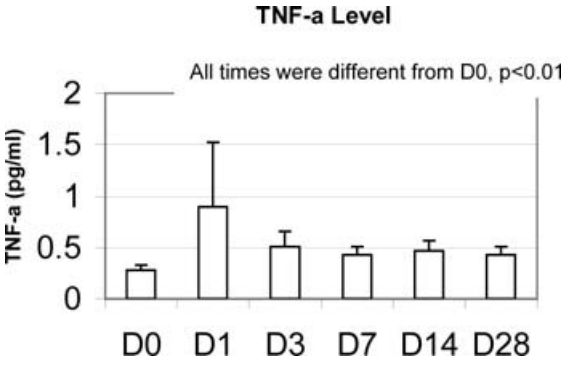

Figure 2. Mean TNF-α concentration at baseline and on days 1, 3, 7, 14, and 28. Even on day 28, the TNF-α concentration was 54% elevated above baseline. Bars indicate standard deviations. Comparisons are to day 0.

tissues, based on the GI, was most severe in the first one or two days after placement of the retraction cord. Under the present experimental conditions, with a relatively plaque-free environment and well-fitting provisional crowns, this injury appeared clinically to reverse itself in 2 weeks. Gingival crevicular flow rate did not reveal significant changes over time. This result is not consistent with Cimason[25] who found that the GCF volume increased with increasing gingival index and pocket depth; however, in the present study there was no significant change in the AL or bleeding index during the 4-week observation period.

While previous clinical and histologic studies support these findings, this study reports a previously unknown elevation in GCF cytokine levels. At the end of the experimental period, 4 weeks after cord placement, the TNF-α levels had not yet returned to baseline for the majority of the patients. These findings suggest that the injury from cord placement may be more significant than previously considered. No medicaments were infused in the cord and the provisional crowns had accurate marginal adaptation. Clinical conditions in which medicaments are placed in the sulcus or the provisional crowns have poor adaptation and attract plaque, which may result in a more severe gingival injury. Under these circumstances it may be likely that the GCF cytokine levels may be higher than those observed in this experiment.

Elevated cytokine levels have been associated with attachment loss. Previous studies have shown that the levels of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α in crevicular fluid and gingival tissue samples are elevated in patients with periodontal disease, smokers, or those with HIV infection.[13,14,19,20] Inflammatory mediators act to elicit important regulatory and feedback mechanisms that determine the disease activity and severity; however, it appears significant that as a result of cord retraction, the TNF-α titer was still 54% elevated over baseline measurement at the end of the 4-week experimental period. While no attachment loss was observed during the 28 days following cord placement, perhaps the time period is too short to observe significant loss. Nevertheless if the cytokine levels continued to be elevated for longer periods of time, attachment loss may occur. It would be of interest to consider the possibility that the gingival recession sometimes observed after crown cementation may not be directly associated with crown contours or cementation, but may be related to the retraction procedure and cytokine levels that remained elevated even after "clinical" healing has occurred. Attachment loss or gingival recession after retraction and delivery of final restoration may compromise the esthetics of the restoration and the periodontal prognosis of the tooth involved[26]; however, this conclusion is speculative since the cytokine levels were monitored only for a 1-month period.

Conclusions

In conclusion, the conflicting findings of several studies including those of Cimason[25] and Gamonal,[27] who reported a relationship between periodontal indices and intracrevicular cytokine levels, suggest that much further work is required to analyze the relationship between GCF cytokine

levels and prosthodontic procedures. In addition to the effects of operative procedures including tooth preparation and gingival retraction, interactions between metallic crowns and the periodontium may cause significant increases in cytokine levels that may be further influenced by fixed prosthodontic operative procedures.[28] Further understanding of the factors that increase intracrevicular cytokine levels may provide strategies for more predictable fixed prosthodontic procedures.

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