The Influence of Restorations and Prosthetic Crowns Finishing Lines on Inflammatory Levels after Non-surgical Periodontal Therapy

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Abstract

Objective: The aim of the present study was to evaluate the inflammatory response in sites where crowns were placed supragingivally, at the level of the gingival margin and subgingivally. These were measured clinically and through the levels of interleukin-1β and matrix metalloproteinase-2, inflammatory mediators, before and after periodontal therapy. **Methods:** From 68 patients analyzed, 10 were selected for this study. The gingival crevicular fluid of the patients was collected and analyzed using standard enzyme-linked immunosorbent assay (ELISA). The clinical parameters were measured and correlated with interleukin-1β and matrix metalloproteinase-2. Both analyses were realized before (baseline) and 2 months after non-surgical periodontal therapy. The two-way variance analysis (two-way ANOVA), Tukey-Kramer multiple comparisons test (post hoc) and Pearson parametric correlation test were performed in statistical analysis. **Results:** There were statistically significant differences before and after non-surgical periodontal therapy when comparing supra- and subgingival margins for the plaque and bleeding indexes ($p < 0.05$). There was a tendency toward correlation between the reduction of plaque index and the reduction of interleukin-1β levels, both for supragingival ($r = 0.694$, $p = 0.026$) and subgingival margins ($r = 0.715$, $p = 0.020$) post non-surgical periodontal therapy. The levels of matrix metalloproteinase-2 were not detectable by ELISA because they were below the detection threshold of the assay. **Conclusion:** Supragingival restorations appeared to be more adequate in promoting periodontal health when compared with the other possible marginal finish lines. They also presented a better response to basic periodontal treatment, according to clinical and inflammatory findings.

Key words: Prosthetic crown margins, restoration margins, biological width, inflammatory mediators, interleukin-1β

Introduction

Biological width in the periodontal tissue comprises the distance from the gingival margin to the alveolar crest (Vacek *et al.*, 1994). Its existence is of prime importance for junctional epithelium adhesion and connective fiber attachment from the gingival tissue to the dental structure (Gargiulo *et al.*, 1961; Lindhe *et al.*, 2005). When a restoration invades the biological space, reestablishing and reorganizing the periodontal tissues involved becomes necessary (Marcum, 1967; Bjorn *et al.*, 1969; Müller, 1986). In many cases, this response has a pathological foundation that may lead to inflammation, pain, and formation of periodontal pockets through attachment and bone loss, both of which are related to different inflammatory signs (Müller, 1986). In this case, some dental restorative procedures may contribute to the installation or progression of periodontal diseases (Mörmann *et al.*,

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1974; Newcomb, 1974), thus making the margins of such restorative procedures one of the most delicate and critical points in this process.

The extensive scientific literature available about dental crown margins focuses mainly on three possible categories: localization, form, and adaptation around the preparation lines. The present study focused on the location of the finishing lines of the restorations, which can be classified as: 1) supragingival, when the preparation margin is above the gingival margin; 2) at the gingival margin, when both the restoration and the gingival margins coincide, and 3) subgingival, when the preparation margin is below the gingival margin (Gardner, 1982).

Supragingival margins are easier to keep clean, and, because they are more visually accessible, they can be more quickly and precisely prepared (Soresen *et al*., 1986; Schantzle *et al*., 2001; Reitemeier *et al*., 2002). However, subgingival margins are the type of preparation most commonly used, even though by clinical and biological standards they seem to be related to some kind of pathological periodontal response, which is substantiated by a change in the periodontal microflora. (Marcum, 1967; Nevins *et al*.,1984). Considering the bacteriological component in the pathogenesis of periodontal disease, an increase in adhesion potential and periodontal pathogenic bacteria colonization may compromise the natural periodontal balance. (Koal *et al.,* 2004). In the light of these facts, the inflammatory response resulting from the interaction between the defense mechanisms of the host organism and the microorganisms from the plaque shall determine the level of tissue destruction.

It is well known that inflammatory processes, and part of the time tissue destruction, are mediated by cytokines, such as interleukin-1 (IL-1). This cytokine is secreted in two molecular forms, α and β (in the extracellular fluid) (Kjeldsen *et al*., 1993), both of which possess post-inflammatory effects and, depending on a series of factors, might induce bone resorption (Van Dyke *et al*., 1993). Similarly, metalloproteinases represent a group of enzymes capable of significantly breaking down the components of the extracellular matrix of the periodontal tissues, participating in the destruction of the connective tissue (Okada *et al*., 1986; Gamonal *et al*., 2000). Matrix metalloproteinase-2 (MMP-2), a fibroblast collagenase, is implicated in the collagen type IV degradation. Considering that the gingival fluid is a plasma-like exudate, which, in its passage from the surrounding tissues to the periodontal pocket, captures mediators involved in destructive tissue responses (Figueredo *et al*., 2001), the analysis of such fluid can contribute to the understanding of the level of inflammation in specific sites.

The aim of the present study was to evaluate the standard inflammatory response in sites where restorations with supragingival margins, at the gingival margin and subgingival margins were placed. In addition, to correlate them with the level of inflammatory mediators IL-1β and MMP-2 found in the gingival crevicular fluid before and two months after non-surgical periodontal therapy

Materials and methods

The study was performed at the University of São Paulo, and it was reviewed and approved by the Institutional Human Research Committee. For the present study, 231 dental records were analyzed, all of which presented data on restorations or dental prosthetic crowns with no marginal excess and supragingival, subgingival and at the crest of the gingival margin preparation lines.

The initial selection phase was carried out through radiographic analysis to confirm the presence of dental prosthetic crowns and restorations, which yielded a total of 68 patient records, or 23% of the total. Following the initial screening, the patients were examined clinically and radiographically and 10 patients were finally selected: 14.7% of the total. All selected subjects provided a written patient consent statement as part of the study protocol.

The following exclusion criteria were also used in the selection process: smoking habit, generalized occlusal trauma, systemically ill patients, pregnant women or patients who were undergoing anti-inflammatory or antibiotic treatment. Locally, the experimental sites were not to exceed 3 mm of depth when probed. The restorations or prosthetic crowns should present no marginal excess and the preparation lines should be supragingival, subgingival or at the gingival level. Healthy teeth were used as the control group; they had no restorations and negative bleeding and plaque indices. Thus, in each selected patient four teeth were used.

Initially, bitewing radiographs were taken to evaluate the study sites of each patient. The clinical parameters taken into consideration for the study were probing depth, clinical attachment level and gingival recession. These were measured with a computerized periodontal probe (Florida Probe®, Florida Probe Corporation, Gainsville, FL) and acrylic guides with pre-established measuring notches at six sites per tooth (mediobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual). The bleeding and plaque indices were analyzed through a dichotomous method. All procedures were carried out by the same operator.

The gingival crevicular fluid was collected using *periopapers* and the quantification of the collected fluids was performed using a *Periotron 6000*. In all collection sessions the device was started 15 minutes prior to the procedure and remained under controlled room temperature to minimize possible interferences in the measurement. The collection procedure was performed in three specific sites, and each one of them represented a different type of finishing line in restorations and/or prosthetic crowns. This procedure

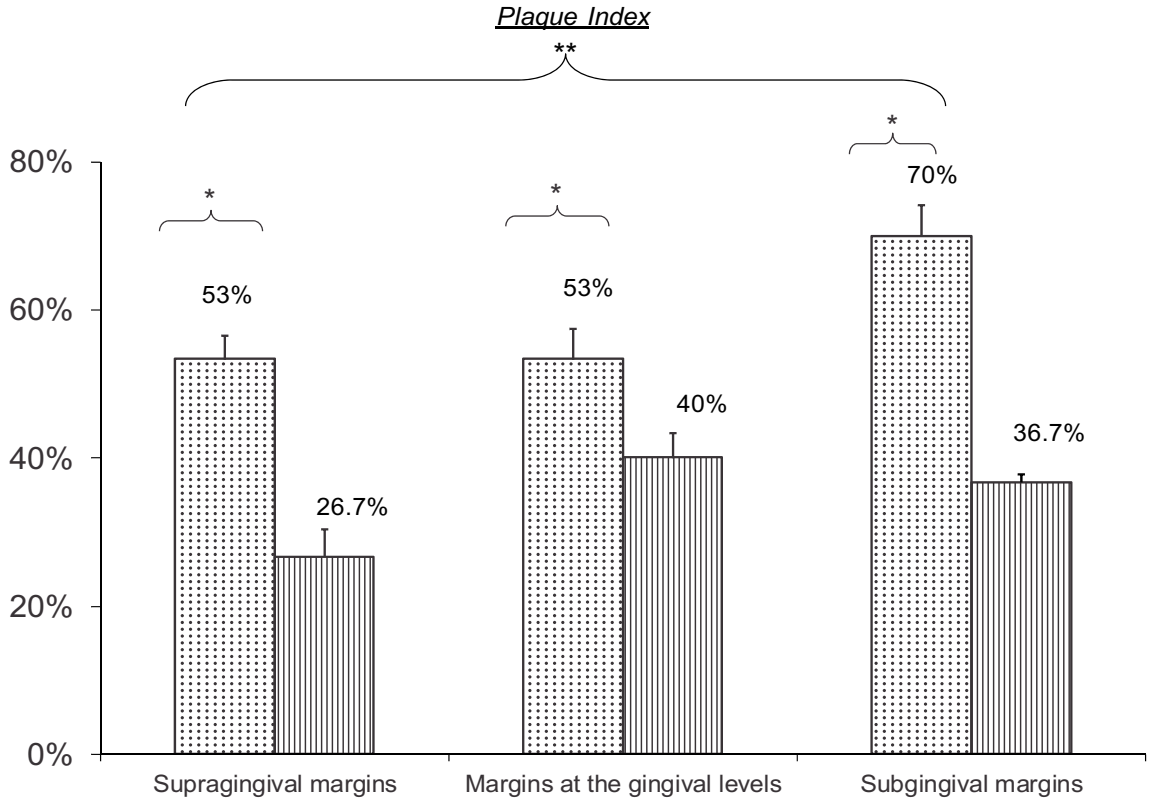

Figure 1. Distribution frequency for the plaque index in supragingival, gingival and subgingival margins at baseline and post non-surgical periodontal therapy (NSPT). *Difference intra-experimental groups, p < 0.05. **Difference among experimental groups, p < 0.05.

was executed at the entrance of the gingival sulcus for one minute in all the patients. For each site, four *periopapers* were embedded for collection and pooled together. Following the collection, the paper cones were taken to the *Periotron 6000* for quantification of the gingival crevicular fluid volume and, after quantified, the *periopapers* were placed in Eppendorf tubes, identified and stored at -80°C until the samples were eluted.

The *Periotron 6000* (Koth, 1977; Jameson, 1979; Koth, 1982) outputs its measurements using a customized measuring unit and, therefore, it became necessary to convert the outputted values from the device to microliters using an appropriate conversion table. The values obtained from the patients, as well as the values converted to microliters, were quantified according to the type of finishing lines; mean values were obtained along with the standard deviations for each one.

After initial data were obtained, the patients underwent non-surgical periodontal therapy in all sextants, which involved prophylactic treatment, supragingival scaling, intra-sulcular scaling with ultrasonic equipment, subgingival scaling when necessary, and detailed instructions on oral hygiene that were repeated every 15 days for two months – the duration of this study. Moreover, recontouring and polishing were carried out at sites that presented excess in restorations margins. At the end of this period, all clinical parameters were re-evaluated and new collection sessions of gingival fluid were performed.

In addition, the gingival crevicular fluid of five patients considered healthy was collected as well. Prophylactic treatment was performed prior to the collection in all sextants to obtain the values to standardize the elution of the samples and the enzyme-linked immunoabsorbent assay (ELISA).

In the present study the sandwich-type ELISA was used, which requires the presence of two antibodies (monoclonal and polyclonal), both of which bind to different epitopes that do not overlap the antigen. Using this method, interleukin-1β (IL-1β) and matrix metalloproteinase-2 (MMP-2) were quantified with human IL-1β and MMP-2 assay kits (Human IL-1β DuoSet kit, Human MMP-2 DuoSet kit, R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. This immunoenzymatic technique involves an *in vitro* reaction of the antigen and antibody, thus being considered a sensitive and specific procedure to detect and quantify proteins – antigens or antibodies (Abba *et al.*, 2004; Vaz *et al.*, 2007).

Therefore, first the *periopapers* were eluted in 450 μL of phosphate-buffered saline (Dulbecco's Phosphate-Buffered Saline, Invitrogen, Gibco, USA) agitated for 30 minutes, aliquoted and stored at -80°C. Two aliquots were used to quantify the inflammatory cytokines through the ELISA method. Briefly, a standard curve was constructed by using standards provided by the

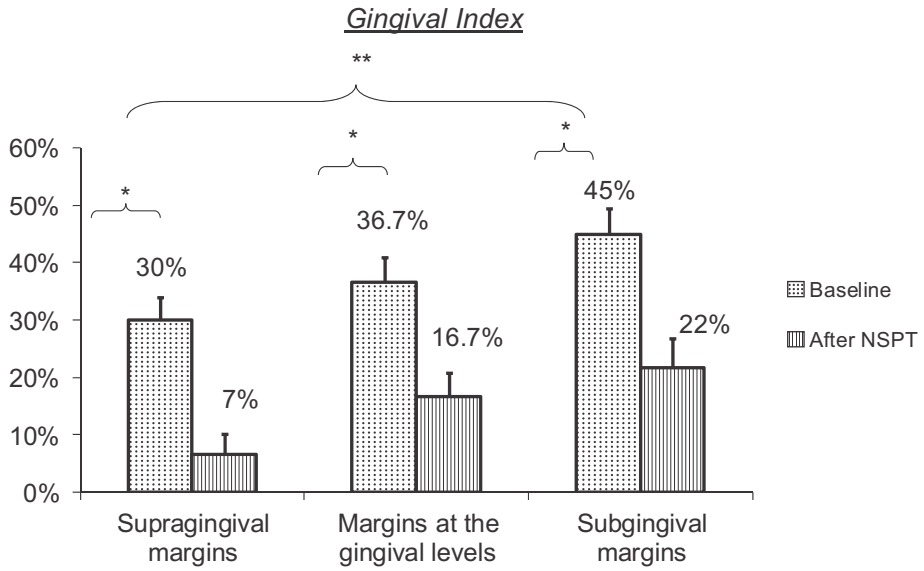

Figure 2. Distribution frequency for the gingival index in supragingival, gingival and subgingival margins at baseline and after non-surgical periodontal therapy (NSPT). *Difference intra-experimental groups, p < 0.05. **Difference among experimental groups, p < 0.05.

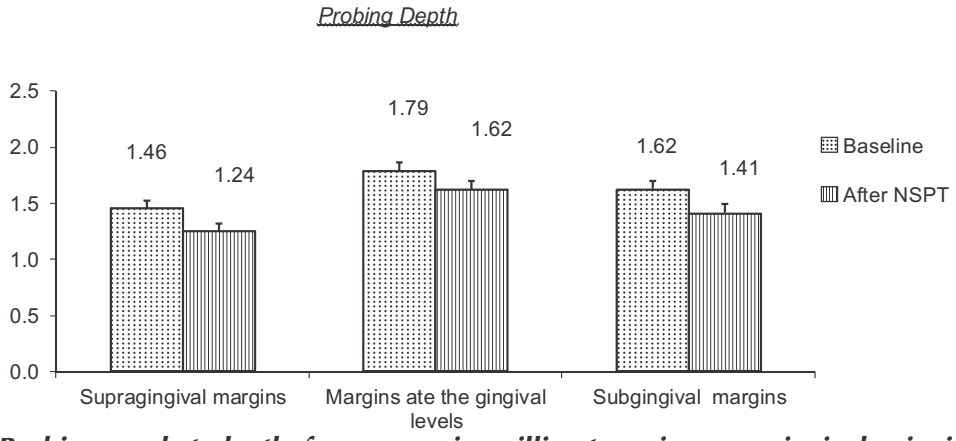

Figure 3. Probing pocket depth frequency, in millimeters, in supragingival, gingival and subgingival margins at baseline and after non-surgical periodontal therapy (NSPT).

kits, and protein concentrations were calculated from the standard curve. A total of 100 μL diluted standard and samples was dispensed, in duplicate, into the wells coated with specific protein antibody. The plate was incubated at room temperature for 1 h, and the wells were washed three times with wash solution. A total of 100 μL conjugate solution (specific protein antibody and peroxidase conjugate) was added, and the plate was incubated at room temperature for 2 h. The wells were washed three times with wash solution, followed by the addition of 100 μL substrate solution. The plate was incubated for 20 minutes at room temperature. The addition of 50 μL stop solution was used to terminate color development. Absorbance was determined by reading the plate at 450 nm. The concentrations of IL-1β and MMP-2 were determined and normalized in picograms/mL (pg/mL) of gingival crevicular fluid.

Each individual was used as an experimental unit for the statistical analysis. Normality tests were conducted so the appropriate test could be chosen. The two-way analysis of variance (ANOVA) and the Tukey-Kramer multiple comparisons test (post hoc) were performed to compare inflammatory cytokines and the clinical parameters observed among the three types of margins before and two months after non-surgical periodontal therapy. The correlation between inflammatory cytokines and the clinical parameters were calculated using the Pearson parametric correlation test. In all statistical analyses a significance level of 5% ($p < 0.05$) was used.

Results

Clinical parameters

The values obtained from the plaque indices, shown in *Figure 1*, were higher the deeper the subgingival finishing lines of the restorations. There was a significant reduction in the plaque index for all the finishing lines between baseline and two months after non-surgical periodontal therapy (NSPT) ($p < 0.001$).

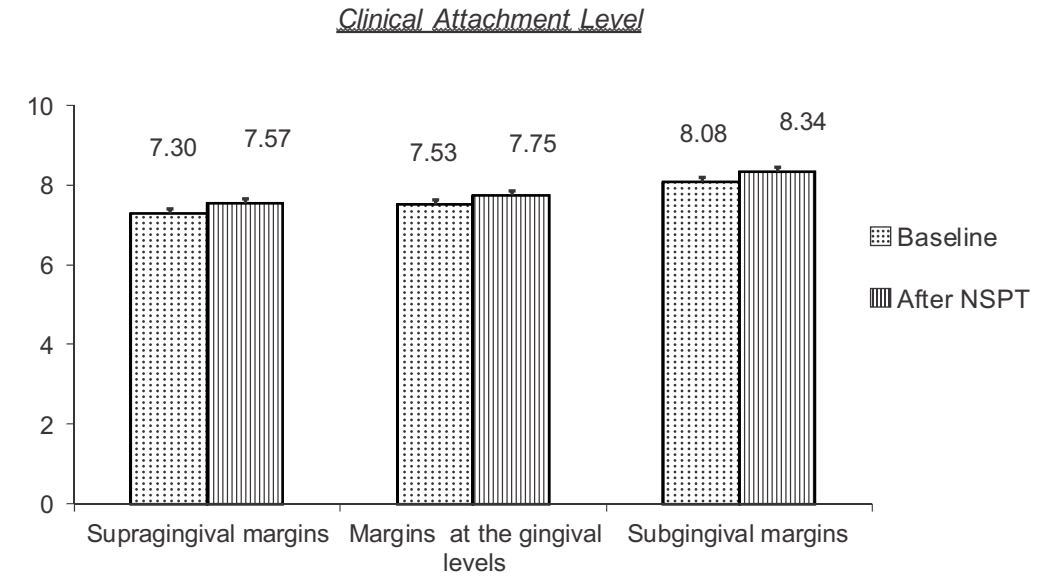

Figure 4. Clinical attachment level, in millimeters, in supragingival, gingival and subgingival margins at baseline and after non-surgical periodontal therapy (NSPT).

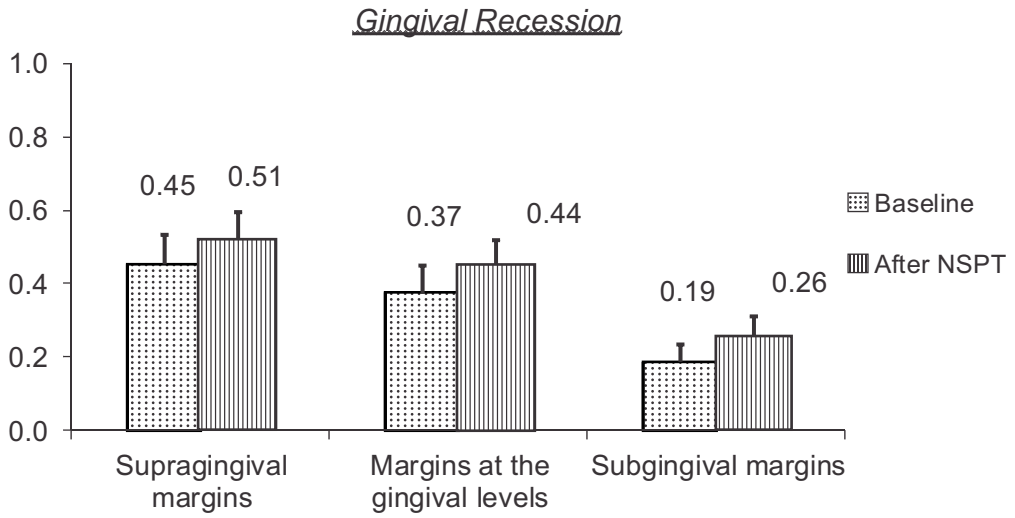

Figure 5. Gingival recession, in millimeters, in supragingival, gingival and subgingival margins at baseline and after non-surgical periodontal therapy (NSPT).

Furthermore, there was a statistically significant difference in the percent reduction of plaque obtained after NSPT between the supragingival and subgingival finishing lines ($p = 0.01$).

Similarly, evaluation of the bleeding index identified that the closer the finishing lines were to the gingival margin, the higher were the measured values. A significant reduction in the bleeding indices for all analyzed finishing lines was found between baseline and post-NSPT ($p < 0.001$, *Figure 2*). A statistically significant difference was also observed for the bleeding index percentage post-NSPT between the supragingival and subgingival finishing lines, in favor of the former ($p = 0.04$).

In terms of probing pocket depth, shown in *Figure 3*, there was no statistically significant difference between baseline and post-NSPT for any of the evaluated margins ($p > 0.05$). Likewise, the reduction obtained for all types of margins did not differ among the types ($p > 0.05$).

There was no statistically significant difference either for the gain in clinical attachment level between baseline and post-NSPT for any of the evaluated margins ($p > 0.05$). Similarly, the gain obtained in all analyzed types of finishing lines did not differ among the types ($p > 0.05$, *Figure 4*).

The increase in gingival recession observed with all types of finishing lines was not statistically significant between baseline and post-NSPT or among all types of finishing lines evaluated ($p > 0.05$, *Figure 5*).

In addition, gingival crevicular fluid was reduced after NSPT for all analyzed groups without any statistically significant differences among them (*Figure 6*).

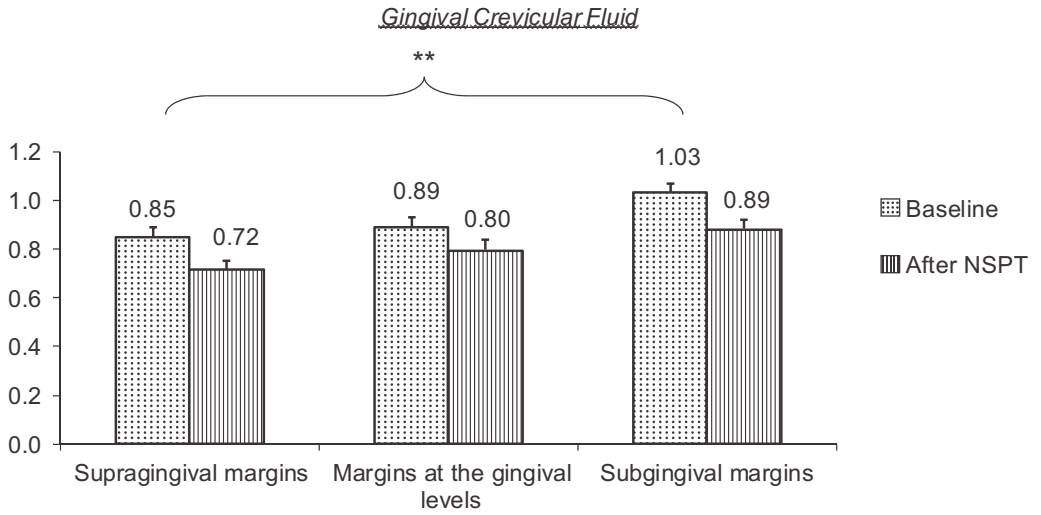

Figure 6. Gingival crevicular fluid level in supragingival, gingival and subgingival margins at baseline and after non-surgical periodontal therapy (NSPT).

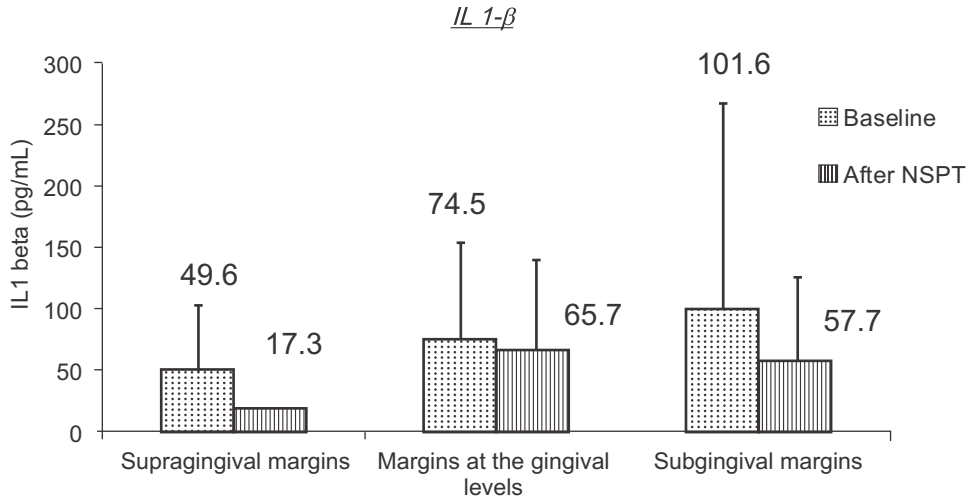

Figure 7. IL-1β levels in supragingival, gingival and subgingival margins at baseline and after non-surgical periodontal therapy (NSPT).

Inflammatory mediators

The values found for IL-1β (*Figure 7*) were lower the more supragingival the finishing lines were positioned. However, these differences were not statistically significant. A reduction in the levels of the analyzed interleukin was also noted before and after non-surgical periodontal therapy for all the groups, but it was not statistically significant either.

The values of MMP-2 were not detectable by ELISA, because they were under the detection threshold of this test.

Correlation between the clinical parameters and IL-1β

Supragingival margin and IL-1β

Examination of the clinical parameters with the values obtained in the analysis of IL-1β revealed a significant correlation between the plaque index post-NSPT and IL-1β (r = 0.694, p = 0.026). In the remaining correlations, there were no statistically significant differences.

Margin at the gingival level and IL-1β

By correlating the clinical parameters with the values found in the analysis of IL-1β, significance between the gingival recession before non-surgical periodontal therapy (baseline) and IL-1β (r = 0.883, p = 0.001) could be demonstrated. In the remaining correlations, there were no statistically significant differences.

Subgingival margin and IL-1β

Finally, the correlation of clinical parameters with the values obtained in the analysis of IL-1β for the subgingival margin showed a significant correlation between the plaque index post-NSPT and IL-1β (r = 0.7155, p = 0.020), and gingival recession post-NSPT

and IL-1β (r = 0.675, p = 0.032). In the remaining correlations, there were no statistically significant differences.

Discussion

The present study has demonstrated that finishing lines of restorations and prosthetic dental crowns, when subgingivally placed, may compromise periodontal health. Thus, higher indices of plaque and bleeding, probing pocket depth and loss of clinical attachment were observed as the margin of the restoration reached a subgingival level.

Periodontal therapy was efficient in the control of plaque and gingival bleeding. All patients, regardless of the finishing lines employed in their restorations or dental prosthetic crowns showed a significant reduction in dental plaque and bleeding indices. Furthermore, this reduction was significantly higher for supragingival margins and much reduced in subgingival ones. Such facts denote that besides the benefits of the applied therapy, other factors also interfered with the patient's plaque control; for example, easier access for their daily hygiene when there were restorations with supragingival finishing lines. Such information corroborates the findings of other studies, in some of which it has been suggested that supragingival margins in restorations are more favorable to periodontal health, not to mention the types of microorganisms associated with it (Richter and Ueno, 1973; Kancyper and Koka, 2001; Reitemeier *et al.*, 2002).

In this study, no statistically significance differences were found in the following parameters: probing pocket depth, clinical attachment level and recession levels. However, it must be noted that only sites that yielded less than 3 mm of probing depth and restorations that did not have adaptation problems were included in this study. Had we included teeth that had deeper probing pocket depths and greater loss of attachment, the larger the reduction in probing depths and the potential respective gain in attachment might have been. Given the selection criterion of shallow pocket depths, such conditions did not reach significant values in the present study.

Restorations placed below the gingival margin are detrimental to gingival health. The degree of inflammation in the gingiva adjacent to a subgingival margin conspicuously exceeded that bordering on tooth surfaces without restorations or with filling margins no closer than 1 mm to the gingival margin. So, violation of the biologic width during preparation may result in an increase in gingival inflammation (Gunay *et al.*, 2000; Schantzle *et al.*, 2001).

The aim of this study was to establish a relationship between the clinical parameters previously discussed and some important inflammatory mediators found in the gingival fluid in periodontal pathologic processes. Initially, it was observed that in the supragingival group the volume of collected gingival fluid was smaller than in the subgingival group. In addition, it was also observed that periodontal therapy reduces the volume of gingival fluid in all types of margins analyzed. Such findings corroborate the premise that the inflammatory process that attacks the periodontal tissues also lead to an increase in the volume of gingival fluid (Jameson, 1979; Shapiro *et al.*, 1980).

A trend towards a correlation between the clinical parameters of plaque index and gingival recession and the evaluated interleukin was observed. This may signify that the percentage of plaque is related to the levels of IL-1β, which in turn are related to the degree of attachment loss. This statement gains particular interest considering that IL-1β possesses pro-inflammatory effects and, depending on a series of factors, it can contribute to bone resorption (Van Dyke *et al.*, 1993). The values found for the interleukin, although more notable in the subgingival margins, were not statistically significant when compared with the other types of margins. The reduction encountered in all groups post non-surgical periodontal therapy was not statistically significant either. For the analysis of this parameter, as expected, there was a great variation in results, which led to a very large standard error, mainly in the more inflamed sites. This error, associated with a small study group, might explain the non-statistically significant differences encountered. The other factor related to this error could be the evaluation time, since two months for evaluation seems to be relatively short. Furthermore, the reduction in the levels of interleukin found after non-surgical periodontal therapy is directly associated with the reduction in the plaque index, and this relationship shows that there was success in the non-surgical periodontal therapy employed.

When it comes to matrix metalloproteinases analysis (MMP-2), no detectable levels were found in the samples. Considering that MMP-2 is an important inflammatory mediator responsible for the destruction of the collagen matrix and is commonly found in large periodontal defects, the selection of sites with a maximum of 3 mm of probing depth might explain the lack of detection.

In conclusion, restoration margins too close to the gingival sulcus lead to an alteration in the periodontal inflammatory aspect even when dealing with sites with probing depths less than 3 mm. Thus, a supragingival margin may be considered less harmful to the periodontal tissues when compared with its counterparts. Moreover, after non-surgical periodontal therapy, a better clinical response could be observed in all types of margins, especially in the supragingival ones.

Acknowledgments

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