Effect of Non-Surgical Periodontal Therapy on C-Telopeptide Pyridinoline Cross-Links (ICTP) and Interleukin-1 Levels

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Background: Biochemical markers harvested from gingival crevicular fluid (GCF) may be useful to identify and predict periodontal disease progression and to monitor the response to treatment. C-telopeptide pyridinoline cross-links (ICTP), a host-derived breakdown product specific for bone, and interleukin-1β (IL-1), a potent bone-resorptive cytokine, have been associated with periodontal tissue destruction. The aim of this study was to examine the effect of non-surgical periodontal therapy on GCF levels of ICTP and IL-1.

Methods: Twenty-five chronic periodontitis subjects were monitored at 8 sites per subject at baseline prior to scaling and root planing and 1, 3, and 6 months after therapy. Four shallow (probing depths <4 mm) and 4 deep (probing depths ≥5 mm) sites were monitored for both marker levels and clinical parameters. GCF was collected for 30 seconds on paper strips, and levels of ICTP and IL-1 were determined using radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) techniques, respectively. Clinical measurements included probing depth (PD), clinical attachment level (CAL), and bleeding on probing (BOP).

Results: Deep sites exhibited significantly (P <0.001) higher ICTP and IL-1 levels compared to shallow sites at all time intervals. ICTP demonstrated a stronger association to clinical parameters than IL-1 including a modest correlation (r = 0.40, P <0.001) between ICTP and attachment loss. Significant improvements in PD, CAL, and BOP were observed at 1, 3, and 6 months in all sites (P <0.01). However, non-surgical mechanical therapy did not significantly reduce ICTP and IL-1 levels over the 6-month period. Further examination of subjects based on smoking status revealed that ICTP levels were significantly reduced at 3 and 6 months and IL-1 levels reduced at 3 months among non-smokers only.

Conclusions: A single episode of non-surgical mechanical therapy did not significantly reduce biochemical markers associated with bone resorption in patients exhibiting chronic periodontitis. Future longitudinal studies are warranted to specifically evaluate the relationship between C-telopeptide pyridinoline cross-links and periodontal disease progression. *J Periodontol 2001;72:1045-1051.*

KEY WORDS

Gingival crevicular fluid/analysis; periodontitis/therapy; pyridinoline cross-links; interleukin-1/adverse effects; bone resorption; disease progression.

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Traditional methods used in the diagnosis of periodontal disease include assessments of gingival inflammation (e.g., bleeding on probing) and periodontal tissue destruction (e.g., probing depth [PD], clinical attachment level [CAL]). Advantages associated with these methods include being easy to use, cost-effective, and relatively non-invasive.[1] However, the major disadvantage of these techniques is that they are static diagnostic parameters, indicating disease history and not current disease status.[2] Therefore, these methods cannot identify highly susceptible patients or actively deteriorating sites. In addition, limitations in measurement reliability necessitate a change of 2 to 3 mm before a site can be identified as having experienced a significant anatomic event.[3,4] Thus, there is a need for the development of new diagnostic tests that can detect the presence of active disease, predict future disease progression, and evaluate the response to periodontal therapy, therefore improving the clinical management of periodontal patients.[5]

A potential new diagnostic involves the direct measurement in the gingival crevicular fluid (GCF) of a bone-specific molecule called pyridinoline cross-linked carboxy-terminal telopeptide of Type I collagen (ICTP). The value of pyridino-

line cross-links as markers of bone turnover stems from their specificity for bone. In skin and other soft tissues, histidine cross-links are the predominant form, and no pyridinoline-like structures exist.[6] The measurement of pyridinoline cross-links is currently used as a diagnostic marker of active bone resorption in metabolic bone diseases such as postmenopausal osteoporosis.[7] The presence of ICTP in GCF has been associated with bone and attachment loss in experimental and natural periodontitis.[8,9] In an experimental periodontitis model, ICTP was found to be highly sensitive and predictive for future radiographic bone loss as measured by computer-assisted digital radiography.[8] More recently, it has been demonstrated that ICTP is elevated in periodontal patients exhibiting high levels of putative periodontal pathogens including *Bacteroides forsythus, Porphyromonas gingivalis, Fusobacterium nucleatum* subspecies, and *Treponema denticola*.[9] Elevated levels of ICTP have also been observed around dental implants colonized by organisms associated with disease progression.[10] Finally, treatment with a matrix metalloproteinase (MMP) inhibitor significantly reduced ICTP levels in patients exhibiting active periodontal disease.[11] The results of these studies suggest that utilization of ICTP as a diagnostic marker may be useful to identify and predict periodontal disease progression and to monitor the response to treatment.

Interleukin-1 (IL-1) is a potent bone-resorbing cytokine formerly known as osteoclast activating factor.[12] Sources of IL-1 include monocytes, neutrophils, fibroblasts, and epithelial cells.[1] IL-1β is the major form secreted by monocytes, and its production is stimulated by bacterial lipopolysaccharide.[13] Once secreted, IL-1 may activate lymphocytes, incite macrophage chemotaxis and prostaglandin production, and stimulate osteoclastic resorption of bone.[14] IL-1 has been detected in both periodontal tissues and GCF in patients with periodontal disease.[15,16] Site-specific elevations have been reported in untreated periodontitis populations and during induction of gingival inflammation.[17-19] Conflicting results have been reported regarding the effect of periodontal treatment on IL-1 levels.[19-21]

The aims of the present study were to examine the effect of non-surgical periodontal therapy (scaling and root planing [SRP]) on GCF levels of ICTP and IL-1 longitudinally and to examine the relationships between ICTP, IL-1, and traditional clinical parameters.

MATERIALS AND METHODS

Study Population

Twenty-five subjects exhibiting moderate to severe chronic periodontitis were recruited for this investigation. Eligible participants had not received any periodontal therapy for a minimum of 6 months and no antibiotic treatment for at least 6 months prior to study inclusion, and were not chronically treated (i.e., 2 weeks or more) with any medication known to affect periodontal status (e.g., phenytoin, calcium antagonists, cyclosporin, coumadin, and non-steroidal anti-inflammatory drugs). Each subject was monitored at 8 sites at baseline (prior to SRP), and 1, 3, and 6 months after treatment. Four shallow (PD <4 mm) and 4 deep (PD ≥5 mm) sites were monitored in each subject for both marker levels (i.e., ICTP, IL-1) and clinical parameters. ICTP and IL-1 levels were determined at different sites within each subject (i.e., 2 shallow/2 deep sites per mediator for a total of 8 sites). Sites sampled for ICTP and IL-1 levels were designated as ICTP sites and IL-1 sites, respectively. After the baseline procedures (i.e., GCF sampling and clinical measurements) were completed, SRP was performed using hand and ultrasonic instrumentation. A maximum of 2 sessions within 14 days with no time limitation was allowed to complete each subject. Local anesthetic was used at the discretion of the clinician. Gingival crevicular fluid sampling (i.e., ICTP, IL-1) and clinical measurements were repeated at 1, 3, and 6 months. No additional therapy was administered over the following 6 months. This study was approved by the Institutional Review Boards at both the University of Michigan and University at Stony Brook, and informed consent was obtained in writing from each subject prior to study commencement.

Clinical Measurements

Clinical measurements were completed at a total of 200 sites (i.e., 25 subjects × 8 sites/subject) at baseline, 1, 3, and 6 months. The parameters recorded included: 1) probing depth (PD) measured from the gingival margin to the base of the pocket in millimeters; 2) clinical attachment level (CAL) measured from either the cemento-enamel junction (CEJ) or restoration margin to the base of the pocket in millimeters; and 3) bleeding on probing (BOP) recorded dichotomously as 0 or 1. All clinical measurements were performed after GCF sampling using a manual probe.§ Five examiners (from 2 test centers) performed all clinical measurements and were calibrated prior to the study to ensure adequate intra- and interexaminer reliability.

Interexaminer reliability was determined using the intraclass correlation coefficient[22] to compare an experienced examiner designated as the gold standard to the 5 examiners participating in this study. The intraclass correlation coefficients for PD and CAL measurements ranged from 0.90 to 0.97 and 0.85 to 0.94, respectively. Intra-examiner reliability was determined utilizing the percent agreement within 1 mm between 2 passes and a weighted kappa statistic. The percent agreement within 1 mm ranged from 95.5% to 97.3%

§ PCP-UNC 15, Hu-Friedy Manufacturing Company, Chicago, IL.

and 90.0% to 95.1% for PD and CAL measurements, respectively. The weighted kappa statistic ranged from 0.92 to 0.97 and 0.88 to 0.92 for PD and CAL measurements, respectively.

Gingival Crevicular Fluid Sampling and Analysis

The area around each site sampled was dried and supragingival plaque removed. Gingival crevicular fluid was collected for 30 seconds using methylcellulose strips.‖ Each strip was placed gently into the gingival crevice until slight resistance was felt and volume determined.¶ Following collection and volume determination, the sample was kept on ice for transport to the laboratory and stored at –20°C. For ICTP, the designated frozen samples were thawed at room temperature and proteins eluted by centrifugation with 20 ml phosphate buffered saline (PBS) 5× at 1,500 × g for 5 minutes. The PBS solution contained 15 nM aprotenin,# 1 mM phenylmethylsulfonylfluoride (PMSF#), and 0.1% human serum albumin (HSA). Levels of ICTP were determined using a radioimmunoassay technique** as previously described.[23] The described technique has been shown to produce >90% efficiency of ICTP recovery.[8]

The IL-1β content was determined by using a commercially available enzyme-linked immunosorbent (ELISA) assay†† that is based on a double antibody sandwich technique, as described by Payne et al.[24]

After the designated IL-1 samples were thawed at room temperature and proteins eluted, plates precoated with a monoclonal antibody specific for human interleukin-1β were incubated with 100 µl of standard or sample and an acetylcholinesterase: anti-IL-1β Fab' conjugate, followed by development with Ellman's reagent. Plates then were read at 405 nm. The assay has a sensitivity of detection of 1.5 pg/ml IL-1β.[24] Both ICTP and IL-1 were reported as total amounts per time of collection (pg/30-second sample).

Statistical Analysis

Clinical parameters and marker levels at various time intervals (i.e., baseline, 1-, 3-, and 6-month visits) were compacted and analyzed using repeated measures analysis of variance. Given significance at the 0.05 level, pairwise comparisons were performed using Fisher's protected t tests. Traditional clinical parameters (i.e., PD, CAL, BOP) were compared to ICTP and IL-1 levels utilizing correlation coefficients that were analyzed for significance using a Fisher's r to z test.

RESULTS

Patient Characteristics

Characteristics of the study group at baseline are presented in Table 1. The cohort comprised 64% females and 48% smokers. The majority of the sites exhibited BOP at initial presentation (71.5%). The baseline PD and CAL measurements ± standard error were 4.27 ± 0.12 mm and 4.30 ± 0.13 mm, respectively. No significant differences (P <0.05) were identified at baseline in regards to PD, CAL, and BOP between sites sampled for ICTP and IL-1. One patient relocated to another area and was not available for the 6-month examination. A total of 792 sites were therefore available for analysis after excluding the 6-month data (8 sites) of one subject.

Table 1.

Baseline Population Characteristics

Age (range)	46.9 years (31.8-68.5)
Females	64.0%
Smokers	48.0%
BOP	71.5%
PD (mean ± SE)	4.27 ± 0.12 mm
CAL (mean ± SE)	4.30 ± 0.13 mm

Table 2.

Clinical Response to Scaling and Root Planing at All Sites

Time	PD ± SE (mm)	CAL ± SE (mm)	BOP ± SE (%)
Baseline	4.27 ± 0.12	4.30 ± 0.13	71.5 ± 3.2
1 month	3.70 ± 0.10*	3.91 ± 0.12*	59.0 ± 3.5†
3 months	3.54 ± 0.09*	3.76 ± 0.11*	53.0 ± 3.5*
6 months	3.66 ± 0.10*	3.94 ± 0.12*	50.0 ± 3.6*

* P <0.0001.
† P <0.01.

Clinical Measurements, ICTP Levels, and IL-1 Levels at Deep and Shallow Sites

Scaling and root planing effects on clinical parameters at all sites are presented in Table 2. Significant improvements in PD, CAL, and BOP were observed at 1, 3, and 6 months in all sites (P <0.01). The measurements recorded for PD, CAL, and BOP stratified for deep/shallow status are presented in Table 3.

Probing depth measurements were significantly reduced at all time intervals following SRP at deep (P <0.0001) and shallow (P <0.04) sites (Table 3). Deep sites also exhibited a significant (P <0.0001) gain in

‖ Pro Flow, Inc., Amityville, NY.
¶ Periotron 6000, Harco Electronics, Tustin, CA.
# Sigma Chemical Company, St. Louis, MO.
** DiaSorin Inc., Stillwater, MN.
†† Cayman Chemical Company, Ann Arbor, MI.

Table 3.

Clinical Response to Scaling and Root Planing at Deep and Shallow Sites

Time	PD ± SE (mm)	CAL ± SE (mm)	BOP ± SE (%)
Baseline			
Deep	5.66 ± 0.13	5.64 ± 0.15	87.0 ± 3.4
Shallow	2.89 ± 0.09	2.97 ± 0.10	56.0 ± 5.0
1 month			
Deep	4.68 ± 0.14*	4.90 ± 0.18*	71.0 ± 4.6‡
Shallow	2.73 ± 0.07†	2.92 ± 0.08	47.0 ± 5.0
3 months			
Deep	4.41 ± 0.13*	4.65 ± 0.16*	70.0 ± 4.6‡
Shallow	2.68 ± 0.08†	2.87 ± 0.09	36.0 ± 4.8‡
6 months			
Deep	4.60 ± 0.14*	4.96 ± 0.16*	60.4 ± 5.0‡
Shallow	2.71 ± 0.06†	2.93 ± 0.08	39.6 ± 5.0‡

* $P<0.0001$.
† $P<0.04$.
‡ $P<0.01$.

CAL after mechanical therapy at all time intervals (Table 3). Deep sites exhibited significant ($P<0.01$) reductions in the percentage of BOP following mechanical therapy. The percentage of BOP at shallow sites was significantly ($P<0.01$) reduced at 3 and 6 months.

The absolute amounts of ICTP (pg/site) and IL-1 (pg/site) collected over 30 seconds at deep and shallow sites are presented in Figures 1 and 2, respectively. Both ICTP and IL-1 levels were significantly greater ($P<0.001$) at sites exhibiting moderate to severe pocket depths. Non-surgical mechanical therapy did not significantly reduce ICTP and IL-1 levels over the 6-month period.

Association of ICTP and IL-1 With Clinical Parameters

The correlations of ICTP and IL-1 with traditional clinical parameters are presented in Table 4. Overall, ICTP exhibited a stronger association than IL-1 to the clinical parameters studied. A modest correlation ($r = 0.40$; $P<0.001$) existed between ICTP levels and clinical attachment loss. Other associations were weak, but significant ($P<0.001$).

Effect of Smoking Status on ICTP and IL-1 Levels

Figures 3 and 4 illustrate ICTP and IL-1 levels, respectively, exhibited in smokers and non-smokers. In non-smoking patients, a significant reduction in ICTP levels was observed at the 3- and 6-month time point ($P<0.02$). A significant reduction in IL-1 levels was observed at the 3-month time point ($P<0.005$). Baseline ICTP levels in non-smokers were significantly higher compared to smokers ($P<0.05$).

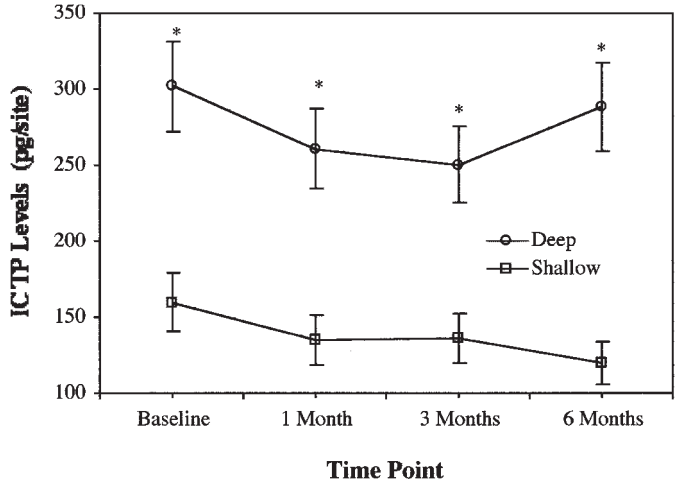

Figure 1.
*Mean ICTP levels ± SE (pg/site) at deep and shallow sites. *Significant differences (P <0.001) observed between deep and shallow sites at all time points.*

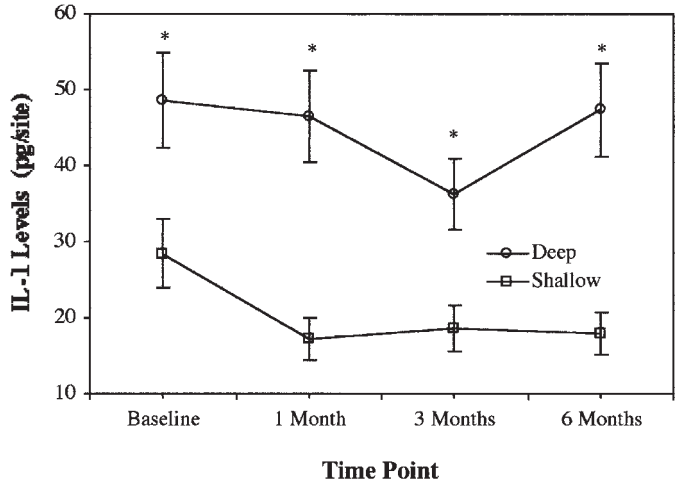

Figure 2.
*Mean IL-1 levels ± SE (pg/site) at deep and shallow sites. *Significant differences (P <0.001) observed between deep and shallow sites at all time points.*

DISCUSSION

Accurate identification of periodontal sites exhibiting disease progression or at risk of future deterioration has proven elusive. Current methods to identify disease progression include 2 probing or radiographic examinations performed over a specific time interval (e.g., 6 months) to detect destructive changes. Therefore, diagnosis of progression occurs only after significant periodontal destruction has transpired. Limitations in measurement reliability also prevent accurate assessment of a patient's response to therapy. Host factors in the GCF associated with the anatomic events of

Table 4.

Correlation of ICTP and IL-1 Levels With Clinical Parameters

Parameter	ICTP	IL-1
PD	0.37*	0.24*
CAL	0.40*	0.22*
BOP	0.22*	0.16*

* Correlation coefficient significant ($P<0.001$).

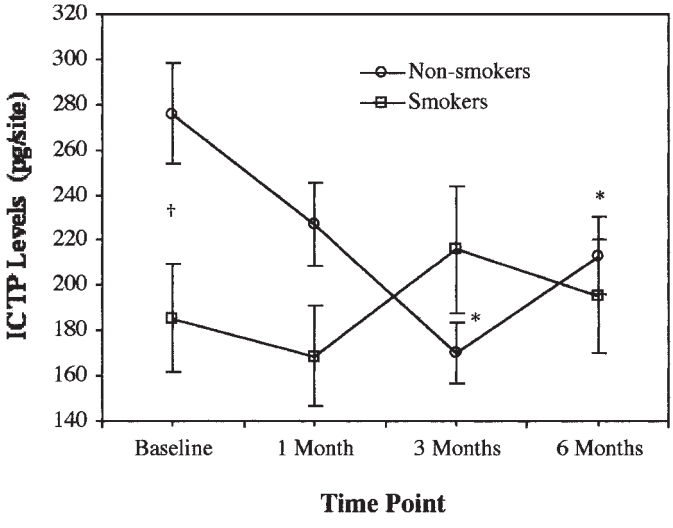

Figure 3.
*Mean ICTP levels ± SE in smokers versus non-smokers. *Significant reduction (P <0.02) in ICTP levels observed at 3 and 6 months in non-smokers only. †Baseline ICTP levels in non-smokers were significantly higher (P <0.05) compared to smokers.*

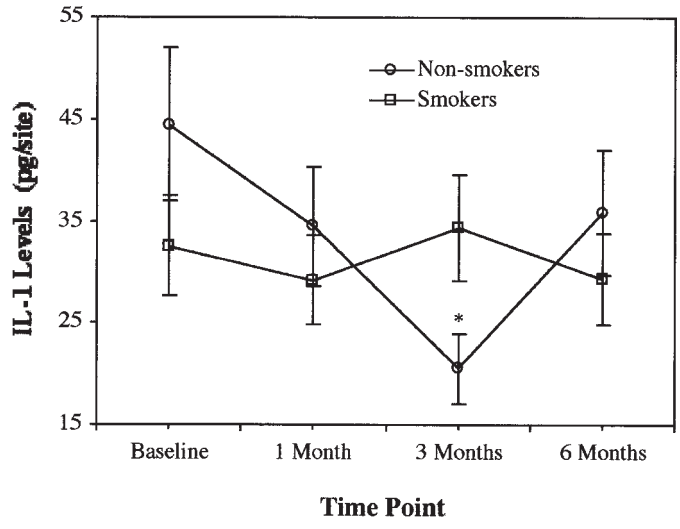

Figure 4.
*Mean IL-1 levels ± SE in smokers versus non-smokers. *Significant reduction (P <0.005) in IL-1 levels observed at 3 months in non-smokers only.*

periodontitis may be useful as markers for identifying the current status of a patient and predicting future disease progression.[25] A biochemical marker specific for bone degradation would assist in the differentiation of gingival inflammation from active periodontal destruction.[26] The results of this longitudinal study demonstrate that higher levels of both a bone-specific marker (ICTP) and a cytokine associated with bone resorption (IL-1) are present at sites exhibiting moderate to severe periodontitis. In addition, utilization of non-surgical mechanical therapy did not significantly reduce these biochemical markers associated with bone resorption over a 6-month period.

Previous reports analyzing ICTP levels in GCF have consistently compared total amounts of GCF versus concentration, and the most predictive results were found when total amounts were normalized by the time of collection.[8-11] The mean total amount of ICTP present at baseline in this cohort of periodontitis subjects was similar to previous investigations that measured ICTP levels in untreated periodontitis subjects.[8,11,27] In contrast, Palys et al.[9] reported lower ICTP levels among a group of previously treated periodontitis subjects participating in a periodontal maintenance program. In addition, these investigators measured ICTP at multiple sites within a subject, providing substantially more healthy than diseased sites for analysis. In the present study, significantly higher ICTP levels were identified at sites exhibiting deep probing depth measurements. Talonpoika and Hämäläinen[27] utilized a similar stratification scheme based upon probing depth and also reported significantly higher ICTP levels among untreated periodontitis subjects at sites with deep PD (i.e., 4 to 10 mm). However, Palys et al.[9] reported no significant differences in ICTP levels at sites exhibiting different PD among a cohort of treated periodontitis patients. These investigators did detect higher levels of ICTP at shallow sites in both a gingivitis and periodontitis cohort compared to periodontally healthy subjects. Overall, ICTP levels were weakly associated to clinical parameters, with periodontal attachment loss demonstrating a modest relationship. The presence of previous attachment loss has been identified as a primary risk factor for periodontal disease progression.[28]

IL-1 levels expressed as absolute amounts were also greater at sites exhibiting significant PD measurements. The positive relationship between absolute amounts of IL-1 and greater PD has been previously reported.[21,29] However, consistent with previous investigations,[21,30] no significant differences were detected between deep and shallow sites in IL-1 levels expressed as a concentration (data not shown). Local concen-

trations of IL-1 and ICTP in GCF varied considerably among subjects and sites, and thus all GCF data were preferably presented on a pg/site basis.

A single episode of non-surgical mechanical therapy did not significantly reduce ICTP or IL-1 levels at any of the monitored time periods. Previous studies utilizing either mechanical[8,27] or chemotherapeutic agents[11] have demonstrated reductions in ICTP levels following periodontal therapy. In the present study, numerically lower ICTP levels were observed at 1 and 3 months following treatment. The lack of significance may be due to the presence of inflammation as evidenced by the high percentage of bleeding on probing sites throughout the 6-month period (Table 3). IL-1 levels also did not diminish significantly following mechanical treatment. Conflicting results have been reported regarding the effect of periodontal treatment on IL-1 levels.[19,21,29] Reinhardt et al.[19] reported no significant differences in IL-1 levels 6 months after scaling and root planing. However, sites in the same cohort treated with periodontal surgery exhibited significant increases in IL-1 levels over the same time period. More recently, Engebretson et al.[30] reported that changes in IL-1 levels following treatment were dependent upon the subjects' composite genotype for the polymorphic IL-1β gene cluster. Significant reductions in IL-1 concentrations after SRP were detected only in subjects who did not possess the periodontitis-associated genotype. Stratification of subjects based upon IL-1 genotype status may be indicated in future clinical trials evaluating novel diagnostic or treatment procedures.

Stratification of subjects by smoking status revealed a different response to scaling and root planing in relation to biochemical mediator levels. No significant differences in ICTP levels were present in smokers post-treatment. However, non-smokers exhibited a significant decrease in ICTP levels at the 3- and 6-month time points ($P < 0.02$) and in IL-1 levels at the 3-month time point ($P < 0.005$). It is well documented that both non-surgical and surgical periodontal therapies are less effective in smokers and recurrence of disease is more likely in smokers after periodontal treatment.[31-34] Interestingly, in the present study, both smokers and non-smokers exhibited similar improvements in clinical parameters following treatment (data not shown). However, it is plausible that the improvements observed in the periodontium at an anatomical level may not represent the metabolic state of the tissues and subsequent risk for disease progression associated with smoking.

The incidence of disease progression among all ICTP sites utilizing a 1 mm and 2 mm threshold were 12% (12/100) and 3% (3/100), respectively. The reduced incidence of disease progression was due to the administration of treatment at the baseline visit. A preliminary analysis was performed to evaluate the association between elevated ICTP levels at baseline and subsequent disease progression (data not shown). Utilizing a site-based analysis and a threshold value of ≥240 pg/site ICTP at baseline, the odds ratio of a site exhibiting ≥1 mm attachment loss at 1 month was 3.7 (C.I. = 1.1 to 12.7; $P = 0.036$). This result suggests that sites with elevated ICTP at baseline were at greater risk of demonstrating attachment loss during the follow-up period. However, the calculation of a subject-based odds ratio to adjust for patient effects was not feasible due to the limited number of sites sampled in each individual. Limitations associated with not controlling for patient effects include the calculation of unreliable Type I and Type II error rates, and failure to obtain a correct assessment of causal relationships between site-specific variables.[35] In a previous study, ICTP was found to be highly sensitive and predictive for future radiographic bone loss as measured by digital radiography.[8] Future longitudinal studies on untreated or maintenance populations are warranted to specifically evaluate the relationship between C-telopeptide pyridinoline cross-links and periodontal disease progression.

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