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Relationship between C-telopeptide pyridinoline cross-links (ICTP) and putative periodontal pathogens in periodontitis

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Abstract

Crevicular fluid pyridinoline cross-linked carboxyterminal telopeptide of type 1 collagen (ICTP) is predictive for future alveolar bone loss in experimental periodontitis in dogs. The present study sought to relate ICTP to a panel of subgingival species in subjects exhibiting various clinical presentations such as health ($n=7$), gingivitis ($n=8$) and periodontitis ($n=21$). 28 subgingival plaque and GCF samples were taken from mesiobuccal sites in each of 36 subjects. The presence and levels of 40 subgingival taxa were determined in plaque samples using whole genomic DNA probes and checkerboard DNA-DNA hybridization. GCF ICTP levels were quantified using radioimmunoassay (RIA). Clinical assessments made at the same sites included: BOP, gingival redness, plaque, pocket depth, and attachment level. Differences among ICTP levels in the 3 subject groups were sought using the Kruskal-Wallis test. Relationships between ICTP levels and clinical parameters as well as subgingival species were determined by regression analysis. The results demonstrated significant differences among disease categories for GCF ICTP levels for healthy (1.1 ± 0.6 pg/site (mean $\pm$ SEM)) gingivitis (14.8 ± 6.6 pg/site) and periodontitis subjects (30.3 ± 5.7 pg/site) ($p=0.0017$). ICTP levels related modestly to several clinical parameters. Regression analysis indicated that ICTP levels correlated strongly with mean subject levels of several periodontal pathogens including *B. forsythus*, *P. gingivalis*, *P. intermedia*, *P. nigrescens* and *T. denticola* ($p<0.01$). The data indicate that there is a positive relationship between the putative bone resorptive marker ICTP and periodontal pathogens.

Keywords

periodontitis; gingival crevicular fluid; periodontal pathogens; bone resorption; pyridinoline cross-links; human studies

One of the limitations in periodontology is the inability to detect actively deteriorating sites in patients susceptible to periodontal disease. Currently, the clinician diagnoses periodontal disease based upon clinical diagnostic aids such as radiographs and probing depth measurements (Armitage 1996). These diagnostics are effective in determining disease status, but are poor predictors of future disease activity (Haffajee et al. 1983, 1991). Recent advances in the diagnosis of metabolic skeletal disorders have lead to the investigation of markers of periodontal alveolar bone loss (Giannobile 1997). Several investigators have implicated bone specific pyridinoline cross-links as markers of bone resorption in periodontitis (Talonpoika &

Hämäläinen 1994, Giannobile et al. 1995, Shibutani et al. 1997) and peri-implantitis (Oringer et al. 1998).

Pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP) is derived from the carboxyterminal telopeptide regions of type I collagen cross-linked via pyridinoline or deoxypyridinoline (Risteli et al. 1993). Following procollagen synthesis and its release into the maturing extracellular matrix, collagen fibrils undergo post-translational modification resulting in cross-links between the telopeptide regions of type I collagen chains by lysyl oxidase. The ICTP cross-link contains two carboxyterminal telopeptides of the α_1 chain and one helical region from the α_2 chain. These cross-links are essential for providing mechanical stability to the maturing matrix and are specific to bone and cartilage and are not found in soft tissues such as skin where the cross-link is initiated by histidine residues (Charles et al. 1994). Elevated serum levels of ICTP have been shown to correlate with bone resorptive metabolic diseases such as primary hyperparathyroidism and post-menopausal osteoporosis (Eriksen et al. 1993, Hassager et al. 1994).

Recently, several studies have been conducted examining C-telopeptides in gingival crevicular fluid (GCF). These investigations have shown that ICTP correlated strongly with radiographic bone level and pocket depth and was significantly higher at periodontitis sites compared to non-periodontitis sites (Talonpoika & Hämäläinen 1994). In a ligature-induced experimental periodontitis study in dogs, the GCF levels of pyridinoline cross-links (ICTP and deoxypyridinoline) significantly increased during the development of attachment loss and osteoclastic bone resorption (Giannobile et al. 1995, Shibutani et al. 1997). Moreover, ICTP was highly sensitive and specific for predicting future alveolar bone loss as measured by computer-assisted digitizing radiography (Giannobile et al. 1995).

Destructive periodontal diseases can be thought of as a series of infections that affect individual or multiple periodontal sites within an individual (Haffajee & Socransky 1994). There is a large body of literature that supports an etiologic role of selected subgingival species such as *Bacteroides forsythus*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum* species and *Treponema denticola* in periodontal diseases (reviewed in Haffajee & Socransky (1994)). These species may promote periodontal tissue destruction, including bone resorption, by the expression of a multitude of virulence factors. The objective of this study was to determine the relationship between GCF ICTP levels and the associated subgingival microbiota in patients with different clinical conditions. This cross-sectional study should allow for the development of future longitudinal investigations.

Material and Methods

Subject population

36 adult subjects were recruited from those seen in the Clinical Center for Periodontal Research at Forsyth Dental Center. All subjects had at least 20 teeth and had received no periodontal treatment or antibiotic-related therapy for medical or dental reasons 3 months prior to the study. In addition, they did not receive long-term use of medications known to affect periodontal status such as anti-inflammatory drugs, aspirin and ibuprofen. The patients had no history of metabolic bone diseases such as rheumatoid arthritis or postmenopausal osteoporosis. 22 subjects were female and 14 were male and ranged in age from 25 to 69 years. 7 were categorized as healthy, 8 as gingivitis and 21 as adult periodontitis. Healthy subjects exhibited <3 mm of attachment loss, no pockets >3 mm, had no radiographic bone loss, and <20 sites with bleeding on probing (BOP). Gingivitis subjects displayed <3 mm of attachment loss, no pockets >3 mm, had no radiographic bone loss, but exhibited >20 sites with BOP. Periodontitis subjects exhibited at least one site with evidence of radiographic bone loss, attachment loss >3 mm, pocket depth (PD) >4 mm and BOP.

Clinical monitoring

The following clinical parameters were measured at 6 sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual, and mesiolingual) for up to 168 sites in each subject: (1) presence of plaque recorded dichotomously as 0 (absence) or 1 (presence); (2) degree of gingival redness recorded dichotomously as 0 or 1; (3) PD measured from the gingival margin to the base of the pocket in mm; (4) attachment level (AL) measured from the CEJ to the base of the pocket in mm; (5) BOP recorded dichotomously as 0 or 1; (6) suppuration recorded dichotomously as 0 or 1. All clinical assessments were made after GCF and microbial sampling. Three examiners performed all clinical measurements and were calibrated prior to the study.

ICTP determination

GCF samples were taken from the mesiobuccal aspect of each tooth in each subject. The area around each sample site was dried with gauze and the supragingival plaque removed. A methylcellulose strip (Pro Flow, Inc., Amityville, NY) was placed into the sulcus until gentle resistance was felt and GCF was collected for 10 s. The GCF volume was determined using a Periotron 6000 (Harco Electronics, Tustin, CA). Following collection and volume determination, the strips were placed immediately into Eppendorf tubes. The samples were subsequently kept on ice for transport to the laboratory and storage at -20°C until needed for analysis.

The frozen samples were thawed at room temperature, followed by elution of proteins by centrifugation in 12×75 ml polypropylene tubes for 5 minutes with 20 μl of a solution of phosphate buffered saline, pH 7.4 containing 15 nM aprotinin (Sigma Chemical Company, St. Louis, MO), 1 mM phenylmethylsulfonylfluoride (PMSE) (Sigma), and 0.1% human serum albumin. This procedure was repeated five times. GCF ICTP levels were quantified using a radioimmunoassay (RIA) (Incstar Inc., Stillwater, MN) as previously described (Risteli et al. 1993). The extraction procedure has shown >90% efficiency in ICTP recovery when tested in trial experiments (Giannobile et al. 1995). ICTP was determined both as total amount/10 s collection (pg/site) and concentration (ng/ml).

Enumeration of subgingival species in plaque samples

After the removal of supragingival plaque, subgingival plaque samples were taken from the mesio-buccal aspect of every tooth (excluding 3rd molars) using individual sterile Gracey curettes. Samples were analyzed for the presence and levels of 40 subgingival taxa (Table 1) using whole genomic DNA probes and checkerboard DNA-DNA hybridization (as described by Haffajee et al. 1997). Signals were evaluated visually by comparison with the standard for the test species. They were recorded as: 0, not detected; 1, $<10^5$ cells; 2, $\sim 10^5$; 3, 10^5 to 10^6 ; 4, $\sim 10^6$; 5 $>10^6$ cells.

Statistical analysis

Data available for each subject included the level and prevalence (% of sites colonized) of 40 test species from up to 28 plaque samples, ICTP levels measured at the same sites as well as clinical data for 6 parameters measured at six sites per tooth. For all analyses, clinical parameters, ICTP levels and individual species were averaged within a subject and then across subjects in the three clinical groups. Differences among groups were sought using the Kruskal-Wallis test. Similarly, ICTP levels at sites with pocket depths <4 , 4–6 and >6 mm were averaged in a subject and then across subjects. Spearman rank correlation coefficients were computed to examine the relationships between ICTP levels and individual species. For these analyses, the outcome variable was the mean ICTP level determined for each subject. The clinical predictor variables included the mean full mouth pocket depth measurement, mean full mouth attachment level measurement and the % of sites in the subjects that exhibited gingival redness,

bleeding on probing and visible plaque. The microbial predictor variables were the % of sites colonized (prevalence) of each species in each subject.

The relationship between clinical category (health, gingivitis or periodontitis) and predictor variables mean full mouth ICTP level and prevalence of bacterial species was examined using stepwise discriminant analysis.

Results

The mean baseline clinical characteristics of the three subject groups are shown in Table 2. Mean PD and mean attachment level (MAL) did not differ significantly between the healthy and gingivitis groups, although the periodontitis group exhibited significantly greater mean PD and MAL than the other two groups ($p<0.01$). Not surprisingly, noticeable differences were seen among the 3 groups with respect to BOP and gingival redness ($p<0.05$). The healthy group displayed minimal BOP while the gingivitis and periodontitis groups displayed comparable levels of BOP. Gingival redness showed a similar trend.

Fig. 1A presents mean ICTP levels for sampled sites in subjects in the three clinical groups. The healthy subjects exhibited nearly undetectable levels of ICTP (1.1 ± 0.6 pg/site; (mean $\pm$ SEM); the gingivitis patients had modest though variable levels (14.8 ± 6.6 pg/site) and the periodontitis patients had significantly elevated and highly variable levels of GCF ICTP (30.3 ± 5.7 pg/site) at ($p=0.0017$). Fig. 1B is a scatter plot of mean ICTP levels for each subject. Interestingly, 75% (6 of 8) of the gingivitis subjects had ICTP levels below that of the mean ICTP levels for periodontitis patients, while all healthy subjects showed consistently low levels of GCF ICTP. When ICTP was measured on a concentration basis (ng/ml), similar trends were seen among the clinical groups (Fig. 2).

Regression analysis indicated a modest statistically significant positive correlation between PD and plaque and mean ICTP levels, while gingival redness, BOP and MAL did not relate with ICTP levels (Table 3). The relationship between ICTP levels and sites in 3 PD categories (<4 , $4-6$, >6 mm) was examined in the three clinical groups. Fig. 3 (left panel) presents the mean ICTP levels at sites with PD <4 mm in the healthy, gingivitis and periodontitis subjects. Shallow sites in both the gingivitis and periodontitis subjects exhibited significantly higher mean ICTP levels than similar sites in healthy subjects. Further, shallow sites in the periodontitis group had higher mean ICTP levels than those of the gingivitis group. However, no significant difference was seen in mean ICTP levels among PD categories in the periodontitis subjects (Fig. 3, right panel).

The relationship between mean ICTP levels and the prevalence (% of sites colonized) of the test species was examined for all subjects using regression analysis. Certain species, including a number of periodontal pathogens such as *B. forsythus* and *P. gingivalis* showed a significant positive correlation with mean ICTP levels (Table 4). Interestingly, regression analysis revealed much stronger relationships between ICTP and selected subgingival species than between ICTP and clinical parameters (Table 3, Table 4).

Table 5 presents the results of stepwise discriminant analysis using prevalence of bacterial species and mean full mouth ICTP levels to predict whether a subject was periodontally healthy, had gingivitis or periodontitis. The first variable selected, prevalence of *P. gingivalis* discriminated periodontitis subjects reasonably well. Addition of ICTP levels improved discrimination of periodontally healthy subjects. The addition of prevalence of *P. intermedia* to the model further improved discrimination resulting in 27 or 36 subjects being correctly classified.

Discussion

The purpose of the present investigation was to examine GCF ICTP levels in subjects with different periodontal conditions and relate ICTP levels to clinical and microbiological parameters in these subjects. The study showed that GCF ICTP was significantly elevated in patients exhibiting periodontal disease compared to gingivitis or periodontally healthy individuals. Further, it was shown that ICTP related modestly to clinical parameters associated with periodontitis. Of greater interest was the strong correlation between ICTP levels and the presence of specific subgingival species including a number of periodontal pathogens. Thus, the results from this cross-sectional investigation suggested a relationship between selected subgingival pathogens and a marker of bone resorption, the C-telo-peptide pyridinoline cross-link (ICTP).

ICTP is a member of a family of pyridinoline cross-links which are specific for osseous and cartilaginous tissues (reviewed in Calvo et al. 1996). The pyridinoline cross-links include free pyridinoline, deoxypyridinoline, the C-terminal and N-terminal telopeptide molecules. Hence, ICTP represents one member of an important group of molecules which are highly associated with bone resorptive diseases, such as postmenopausal osteoporosis. The release of pyridinoline cross-links into urine or blood can be reversed by estrogen replacement or bisphosphonate therapy which inhibit osteoclastic bone resorption (Garnero et al. 1994, Seibel et al. 1994). When released from the extracellular matrix of mineralized tissues such as alveolar bone, pyridinoline cross-links can be detected in the GCF by immunological methods. Therefore, ICTP may be a first generation pyridinoline cross-link detectable in GCF. Newer methods may involve the use of deoxypyridinoline and other cross-links as immunodetection methods are optimized.

The results of this investigation extend previous studies (Giannobile et al. 1995, Talonpoika & Hämäläinen 1994) that demonstrated a relationship between ICTP and inflammatory periodontal disease. Shibutani et al. (1997) showed similar findings with deoxypyridinoline (found most abundantly in bone and dentin) measured in the GCF, serum and urine during experimental periodontitis in dogs. The initiation of osteoclastic bone destruction and the corresponding elevation in deoxypyridinoline could be detected within 3 days of disease induction by immunohistochemical staining of TRAP+ osteoclast-like cells. Therefore, pyridinoline cross-links may be useful for predicting future bone loss in the periodontium.

This investigation revealed variability in ICTP levels within periodontitis subjects, as well as at individual sites within periodontitis patients (data not shown). Further, the concentration and total amounts of ICTP from periodontitis patients were less than previously described (Giannobile et al. 1995, Golub et al. 1997, Talonpoika & Hämäläinen 1994). This finding may be due, in part, to the patient population studied. The majority of subjects were in a periodontal maintenance program. In addition, multiple sites within a subject were evaluated for ICTP, providing substantially more healthy than diseased sites for analysis. Periodontal therapy has been shown to diminish ICTP levels. For example ICTP levels decreased dramatically following mechanical therapy (Giannobile et al. 1995, Talonpoika & Hämäläinen 1994) or systemically administered doxycycline (Golub et al. 1997). In this last study, 18 patients with moderate to severe periodontitis demonstrated potent reductions in GCF ICTP levels during twice daily administration of 20 mg doxycycline for 2 months. ICTP levels decreased nearly 70% following the first month of administration. This reduction in GCF ICTP was maintained for an additional month. Interestingly, osteocalcin, a marker of bone turnover did not relate to ICTP and did not predict response to therapy. Levels of matrix metalloproteinases (MMPs)-8 and -13 were decreased concomitant with ICTP reductions during the 2 month observation period (Golub et al. 1997). These data suggest that MMP inhibitors, such as low dose

doxycycline, may block attachment and alveolar bone loss resulting from host response modifiers such as MMPs.

The levels of cytokines and tissue degradative components in the GCF have been related to subgingival organisms. (Hirose et al. 1997, Lee et al. 1995, Smith et al. 1997). Inflammatory cytokines such as IL-1 β , IL-6 and TNF- α have been shown to relate strongly with subgingival organisms such as *P. intermedia* in refractory periodontitis (Lee et al. 1995) and *P. gingivalis* fimbriae in adult periodontitis patients (Hirose et al. 1997). Smith et al. (1997) demonstrated the relationship between *P. gingivalis* and the extracellular matrix glycosaminoglycan chondroitin-4-sulfate (C4S) in periodontitis sites. C4S was elevated in *P. gingivalis*-infected sites compared to *P. gingivalis*-free sites. In addition, BANA positive sites, indicative of trypsin-like activity due to species such as *B. forsythus*, *P. gingivalis* and *T. denticola*, possessed >6 fold higher levels of C4S than BANA negative sites (Smith et al. 1997). The data from the current investigation revealed similar relationships between *B. forsythus*, *P. gingivalis* and *T. denticola*, identified by checkerboard DNA-DNA hybridization, and increased ICTP levels.

Socransky et al. (1998) have recently described microbial complexes in subgingival plaque. One complex ("red complex") consisted of *B. forsythus*, *P. gingivalis* and *T. denticola*. These organisms are found frequently together in advanced periodontitis lesions (Gmur et al. 1989, Simonson et al. 1992, Umeda et al. 1996) and are considered to be periodontal pathogens (Zambon 1996). The data from the present investigation indicate that these species relate significantly to increased GCF ICTP levels. Other organisms which also relate to increased ICTP levels are members of a second complex ("orange complex") and included *Fusobacterium*, *Prevotella* and *Campylobacter* species. These organisms are capable of producing a wide range of virulence factors which can directly or indirectly affect alveolar bone (Gopalsami et al. 1993, Slots & Genco 1984, Socransky & Haffajee 1991, Tanner et al. 1985). Under appropriate conditions, certain subgingival species may stimulate a host response that can lead to cytokine release, induction of osteoclastic activity and cleavage of pyridinoline cross-links in bone collagen. Future longitudinal studies should evaluate the relationship between the pathogens that initiate periodontal disease, the cytokines involved in tissue destruction (e.g., IL-1 β , TNF- α and PGE₂) and the resultant pyridinoline cross-link release. A better understanding of the pathogenesis of periodontitis may lead to the development of improved strategies for the diagnosis of periodontal bone loss.

Zusammenfassung

Der Zusammenhang zwischen C-Telopeptid Pyridinolin Quervernetzungen (ICPT) und mutmaßlichen Pathogenen bei der Parodontitis

Das mit Pyridinolin quervernetzte carboxyterminale Telopeptid des Typ I Kollagens (ICTP) im krevikulären Sekret, ist für den zukünftigen Schwund des Alveolatknochens bei experimenteller Gingivitis in Hundever-such von prädiktiver Bedeutung. Die vorliegende Studie versuchte ICTP in Beziehung zu einer Gruppe subgingivaler Mikroorganismen von Personen mit unterschiedlichen klinischen Erscheinungsbildern, wie Gesundheit ($n=7$), Gingivitis ($n=8$) und Parodontitis ($n=21$) zu bringen. Bei jeder der 36 Versuchspersonen wurden an mesiobukkalen Stellen 28 subgingivale Plaque- und GCF Proben entnommen. In den Plaqueproben wurde das Vorkommen und die Zellzahl von 40 subgingivalen Bakterien-Taxa mittels genomischen DNS Sonden und Checkerboard DNA-DNA Hybridisierung ermittelt. GCF ICTP Level wurden durch Radioimmunoassay (RIA) quantifiziert. Klinische Befundungen an den gleichen Stellen galten für: BOP, Rötung der Gingiva, Plaque, Taschentiefe und Attachmentlevel. Unterschiede zwischen den ICTP Level in den 3 Versuchsgruppen wurden mit dem Kruskal-Wallis Test ermittelt. Der Zusammenhang

zwischen den ICTP Mengen und klinischen Parametern, wie auch den subgingivalen Species wurde mit einer Regressionsanalyse bestimmt. Hinsichtlich CGF ICTP Level bei gesunden (1.1 ± 0.6 pg/Stelle (Mittelwert $\pm$ SEM)), an Gingivitis (14.8 ± 6.6 pg/Stelle) und an Parodontitis erkrankten Versuchspersonen (30.3 ± 5.7 pg/Stelle) ($p=0.0017$), machten die Ergebnisse signifikante Unterschiede zwischen den Krankheitskategorien deutlich. Bei den ICTP Level konnten nur mäßige Beziehungen zu einzelnen klinischen Parametern konstatiert werden. Die Regressionsanalyse zeigte, daß die ICTP Level mit den Durchschnittszellzahlen gewisser parodontaler Pathogene der Probanden, speziell *B. forsythus*, *P. gingivalis*, *P. intermedia*, *P. nigrescens* und *T. denticola* ($p<0.01$), stark korrelierte. Die Ergebnisse lassen erkennen, daß ein positiver Zusammenhang zwischen dem mutmaßlichen Marker der Knochenresorption ICTP und parodontalen Pathogenen vorliegt.

Résumé

Relation entre les télopeptides-C avec liaisons croisées via la pyridinoline et des pathogènes parodontaux putatifs dans la parodontite

Régions télopeptides carboxyterminales du collagène type I avec liaisons croisées via la pyridinoline ou la deoxypyridinoline=ICTP. L'ICTP présente dans le fluide crévicaire prédit la perte osseuse alvéolaire dans la parodontite expérimentale chez le chien. L'étude présente essaye de mettre en relation l'ICTP avec plusieurs espèces sous-gingivales chez des sujets avec saut gingivale ($n=7$), gingivite ($n=8$) ou parodontite ($n=21$). 28 échantillons de plaque dentaire sous-gingivale et de fluide crévicaire ont été prélevés de sites mésiovestibulaires chez 36 sujets. La présence et les teneurs de 40 types sous-gingivaux ont été déterminées dans ces échantillons de plaque dentaire en utilisant les sondes ADN et l'hybridisation ADN-ADN en damier. Les niveaux d'ICTP dans le fluide crévicaire ont été quantifiés en utilisant l'essai radio-immunitaire. Les évaluations cliniques faites au niveau des mêmes sites comprenaient la plaque dentaire, la rougeur gingivale, la profondeur de poche au sondage, le niveau d'attache, le saignement au sondage et la suppuration. Les différences parmi les niveaux d'ICTP parmi les trois groupes de sujets étaient analysées en utilisant le test de Kruskal-Wallis. Les relations entre les niveaux d'ICTP et les paramètres cliniques ainsi que les espèces sous-gingivales étaient déterminées par l'analyse de régression. Les résultats ont mis en évidence des différences ($p=0.0017$) parmi les niveaux d'ICTP dans le fluide gingival: santé gingivale (1.1 ± 0.6 pg/site (moyenne $\pm$ SEM)), gingivite (14.8 ± 6.6 pg/site) et parodontite (30.3 ± 5.7 pg/site). Les teneurs en ICTP avaient une relation modeste avec les différents paramètres cliniques. L'analyse de régression a indiqué que les niveaux d'ICTP étaient en forte relation ($p<0.01$) avec les niveaux moyens par sujet de diverse pathogènes parodontaux comprenant le *B. forsythus*, le *P. gingivalis*, le *P. intermedia*, le *P. nigrescens* et le *T. denticola*. Ces données indiquent une relation positive entre le marqueur ICTP putatif de la résorption osseuse et des pathogènes parodontaux.

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Fig. 1.

Panel A is a bar chart of mean ($\pm$ SEM) ICTP levels (pg/site) in healthy, gingivitis and periodontitis subjects. The numbers above the bars represent the number of sites analyzed in each group. ICTP levels were averaged for subject and then across subjects in the three groups. Significance of differences were sought using the Kruskal-Wallis test. Panel B is a scatter plot of mean ICTP levels for each subject in the three subject groups. Each circle represents the mean ICTP level measured at >20 sites in each subject. The horizontal lines represent mean ($\pm$ SEM) ICTP levels for each group.

Fig. 2. Bar chart of the mean ($\pm$ SEM) ICTP concentrations (ng/ml) in the healthy, gingivitis and periodontitis groups. Computation of mean values and the statistical analysis were performed as described for Fig. 1. The numbers above the bars represent the number of sites analyzed in each subject group.

Fig. 3. Bar chart of mean ($\pm$ SEM) ICTP levels (pg/site) at sites with different PDs. Panel A shows mean ICTP levels at shallow sites (<4 mm) in the three subject groups. ICTP levels were averaged for each PD category within a subject and then averaged across subjects for each PD group. Significance of differences was tested using the Kruskal-Wallis test. Panel B is a bar chart of mean ICTP levels at pockets <4 mm, 4–6 mm and >6 mm in the periodontitis subjects. Computation of mean ICTP levels and statistical analysis were as described above.

Table 1
DNA probes used to examine subgingival plaque samples

<i>Actinobacillus actinomycetemcomitans a</i>	<i>Fusobacterium nucleatum ss nucleatum</i>
<i>Actinobacillus actinomycetemcomitans b</i>	<i>Fusobacterium nucleatum ss polymorphum</i>
<i>Actinomyces naeslundii 2</i>	<i>Fusobacterium nucleatum ss vincentii</i>
<i>Actinomyces odontolyticus</i>	<i>Fusobacterium periodonticum</i>
<i>Bacteroides forsythus</i>	<i>Peptostreptococcus micros</i>
<i>Bacteroides fragilis</i>	<i>Porphyromonas endodontalis</i>
<i>Bacteroides ureolyticus</i>	<i>Porphyromonas gingivalis</i>
<i>Campylobacter concisus</i>	<i>Prevotella intermedia</i>
<i>Campylobacter curvus</i>	<i>Prevotella nigrescens</i>
<i>Campylobacter gracilis</i>	<i>Selenomonas noxia</i>
<i>Campylobacter rectus</i>	<i>Streptococcus constellatus</i>
<i>Campylobacter showae</i>	<i>Streptococcus gordonii</i>
<i>Campylobacter sputorum ss bubulus</i>	<i>Streptococcus intermedius</i>
<i>Campylobacter sputorum ss sputorum</i>	<i>Streptococcus mitis</i>
<i>Capnocytophaga gingivalis</i>	<i>Streptococcus oralis</i>
<i>Capnocytophaga ochracea</i>	<i>Streptococcus sanguis</i>
<i>Capnocytophaga sputigena</i>	<i>Streptococcus sp.</i>
<i>Capnocytophaga sp.</i>	<i>Treponema denticola</i>
<i>Eikenella corrodens</i>	<i>Veillonella parvula</i>
<i>Eubacterium nodatum</i>	<i>Wolinella succinogenes</i>

Table 2Mean ($\pm$ SD) baseline clinical characteristics of the subject groups

	Health (n=7)	Gingivitis (n=8)	Periodontitis (n=21)
age (years)	28 $\pm$ 3	39 $\pm$ 13	43 $\pm$ 9
no. missing teeth **	0.9 $\pm$ 16.	2.4 $\pm$ 2.8	2.8 $\pm$ 2.0
% males	29	25	48
mean pocket depth (mm) **	2.24 $\pm$ 0.30	2.37 $\pm$ 0.17	3.18 $\pm$ 0.48
mean attachment level (mm) **	1.60 $\pm$ 0.57	1.97 $\pm$ 0.46	3.04 $\pm$ 0.79
% sites with:			
Plaque	45 $\pm$ 25	40 $\pm$ 35	63 $\pm$ 28
gingival redness	49 $\pm$ 32	76 $\pm$ 19	77 $\pm$ 23
BOP*	6 $\pm$ 5	21 $\pm$ 14	27 $\pm$ 16
suppuration	0 $\pm$ 0	0 $\pm$ 0	1.2 $\pm$ 3.3
N ICTP samples	145	163	486

* $p < 0.05$, Kruskal-Wallis test.** $p < 0.01$.

Table 3

Relationship between mean clinical parameters and mean subjects ICTP levels

Clinical parameter	Spearman rank correlation coefficient
pocket depth *	0.41
plaque *	0.40
gingival redness	0.30
attachment level	0.27
BOP	0.20

* Significant at $p < 0.05$.

Table 4

Relationship between prevalence of subgingival species and mean subject ICTP levels

Species *	Spearman rank Correlation coefficient
<i>C. rectus</i>	0.55
<i>B. forsythus</i>	0.54
<i>F. periodonticum</i>	0.53
<i>C. schowae</i>	0.52
<i>P. intermedia</i>	0.49
<i>F. nucleatum ss nucleatum</i>	0.48
<i>P. nigrescens</i>	0.47
<i>E. nodatum</i>	0.46
<i>P. gingivalis</i>	0.42
<i>F. nucleatum ss polymorphum</i>	0.42
<i>T. denticola</i>	0.40

* All species significant at $p < 0.01$.

Table 5
Predicted and actual classification of subjects based on 1, 2 or 3 predictor variables: H = periodontally healthy, G = gingivitis, P=periodontitis; the predictor variables were selected using stepwise discriminant analysis

	Actual			P		
	H	G		H	G	
Predicted						
H	0	2		7	5	2
G	6	6		2	2	6
P	1	0		12	1	13
	<i>P. gingivalis</i>			<i>P. gingivalis</i>		<i>P. gingivalis</i>
				ICTP		ICTP
						<i>P. intermedia</i>