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Interleukin-1β, tumor necrosis factor-α levels and neutrophil elastase activity in peri-implant crevicular fluid

Correlation with clinical parameters and effect of smoking

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Abstract: The aim of this study was to determine interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) levels and neutrophil elastase (NE) activity in peri-implant crevicular fluid (PICF) of smoker and nonsmoker patients, and to investigate their relationships with clinical parameters. A total of 42 endosseous root-form dental implants of 14 patients were clinically examined by modified Plaque index (PI), modified Gingival index (GI) and probing depth (PD). Smoking habits of the patients were recorded. PICF of implants were collected by Periopaper strips and IL-1β, TNF-α levels were determined by enzyme-linked immunosorbent assay (ELISA). NE was analyzed with a neutrophil specific chromogenic substrate, *N*-methoxysuccinyl-Ala-Ala-Pro-Val-p-nitroanilide. The cytokine and enzyme levels in PICF were expressed as total amount/activity and as concentrations. NE activity in PICF significantly correlated with GI and PD, and IL-1β levels with GI and PICF volume ($P < 0.05$). The correlations were stronger when the PICF levels were expressed as total IL-1β amount and as total NE activity. The implants with inflamed gingiva (GI $>$ 1) had higher levels of IL-1β and NE activity than implants with noninflamed or slightly inflamed gingiva (GI $\leq$ 1) ($P < 0.05$). Total NE activity in implants with deep pockets (PD $>$ 3 mm) was greater than the implants with shallow pockets (PD $\leq$ 3 mm) ($P < 0.05$). The implants of smoker patients had significantly lower PICF NE activity and IL-1β levels, and significantly higher TNF-α levels than the implants of nonsmokers ($P < 0.05$). The findings of the present study indicate that NE activity and IL-1β levels in PICF may be used to measure implant health status of patients who do not smoke.

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It has been well established that following osseointegration and loading, failing implants develop peri-implant inflammation similar to periodontitis; progressive peri-implant bone loss is defined as peri-implantitis (Meffert et al. 1992). Clinical signs such as soft tissue inflammation, bleeding on probing, suppuration, pain, increased probing depth, and radiographic evidence of bone loss (Lekholm et al. 1986a), as well as microbiologic alterations in the flora (Lekholm et al. 1986b; Mombelli et al. 1987), mimic those signs associated with periodontal disease. In an immunohistochemical study, it has been demonstrated that peri-implant inflamed mucosa biopsies comprises mainly lymphocytes, macrophages and only very few plasma cells similar to inflamed gingival tissue (Seymour et al. 1989).

Recent evidence indicates that inflammatory cytokines, released by the host's monocytes and macrophages in response to bacterial products such as lipopolysaccaride and endotoxin, are responsible for the breakdown of the periodontium in periodontitis (Jandinski et al. 1991; Stashenko et al. 1991a). Some studies (Stashenko et al. 1991b; Figueredo et al. 1999) have previously shown that interleukin-1β (IL-1β) is

present at elevated levels in the gingival crevicular fluid and in tissue from periodontal pockets. Tumor necrosis factor-α (TNF-α), a cytokine with functions similar to those of IL-1β, has been detected in very low levels from sites with periodontitis. These cytokines stimulate bone resorption, prostaglandin synthesis and protease production by many cell types including fibroblasts and osteoblasts (Beutler & Cerami 1986; Dewhirst et al. 1985; Billingham 1987; Lorenzo et al. 1987; Tatakis et al. 1988).

During gingivitis and periodontitis, high levels of proteolytic enzymes can be found both in the gingival tissues and in the sulcus. Connective tissue degradation in periodontal disease is believed to result from the action of proteases. Among the proteases, elastase and collagenase have received a great deal of attention during the last decade. Neutrophil elastase (NE) is a neutral serine protease stored in the azurophilic granules of granulocytes. This enzyme degrades, besides elastin, several functionally and structurally important molecules of the periodontium including collagen, proteoglycans and basement membrane components (Cergneux et al. 1982; Watanabe et al. 1990). It has been shown that gingival crevicular fluid elastase activity correlated with clinical parameters of periodontal disease severity (Eley & Cox 1992a) and probing attachment and bone loss (Eley & Cox 1992b) in untreated periodontitis patients.

Although extensive research has been done in the area of periodontal inflammatory mediators, few have studied the role of the host immune response of peri-implantitis. Peri-implant crevicular fluid (PICF) NE levels have been found significantly high around failing implants when compared to healthy implants (Boutros et al. 1992). In mouths with failing implant sites, significantly elevated PICF levels of IL-1β have been determined as compared to mouths with healthy control implants (Panagakos et al. 1996; Salcetti et al. 1997). The authors suggested that NE may be a risk marker for peri-implantitis and that IL-1β may be used to monitor disease progression.

It has been well demonstrated that cigarette smoking has a significant impact on the risk for developing periodontal disease. Both the incidence and the severity of periodontitis is greater in smokers than in nonsmokers (Beck 1994; Bergström & Preber 1994; Grossi et al. 1995). Furthermore, smokers do not respond to periodontal treatment as well as nonsmokers, reflecting the general impairment of the tissue repair process (Ah et al. 1994; Preber et al. 1995). In the literature, studies evaluating smoking as a risk factor for peri-implant disease are lacking. However, Bain & Moy (1993) have reported that the percentage of implant failures was greater in smokers than in nonsmokers. It has also been demonstrated that smoking is a risk factor for implant loss with a relative risk of 2.5 compared to nonsmokers (Wilson & Nunn 1999).

Recent advances in the understanding of biologic events involved in the pathogenesis of periodontitis indicate that bone-resorbing cytokines IL-1β and TNF-α, as well as NE, may also be operative in the pathogenesis of peri-implantitis. With this background, the present study aimed to determine IL-1β, TNF-α levels and NE activity in PICF, and to explore their relation with clinical parameters. This study also evaluated the levels of these cytokines and enzyme activity regarding smoking habits of patients.

Material and methods

Subject selection

Fourteen partially edentulous patients (eight men, six women) who had been treated with endosseous dental implants and were receiving maintenance care participated in this study. Patient ages ranged from 35 to 60 years, with a mean of 49.7 years. Patients were included if they had at least one root-form implant restored and in function for at least 1 year. Subjects were excluded from the study if they were not medically healthy and/or were taking medication that could influence peri-implantitis. These included patients requiring antibiotic prophylaxis and those who had used 0.12% chlorhexidine rinse, systemic antibiotics, cortisone, or nonsteroidal anti-inflammatory drugs within the past 6 weeks prior to clinical examination and crevicular fluid sampling. Patients who had undergone any periodontal or peri-implant therapy within the last 6 months were also excluded from the study. The smoking habits of the patients were evaluated and regular daily cigarette smokers with current consumption more than 10 cigarettes per day were recorded as smokers. Informed consent was obtained in writing from each subject.

Clinical measurements

Prior to crevicular fluid collection, supragingival plaque was scored using modified Plaque index (PI) (Mombelli et al. 1987) and removed from each implant. Gingival inflammation was scored following crevicular fluid collection using modified Gingival index (GI) (Apse et al. 1989). Probing depth (PD) measures were obtained from sampled sites (mesial and distal midpoints) of implants using a conventional periodontal probe (Hu-Friedy, Chicago, IL, USA). The probe was directed parallel to the long axis of the implant. Radiographic bone loss was evaluated on periapical radiographs. All clinical data were collected by one examiner.

Crevicular fluid sampling

The individual implant site was gently air-dried and the area was carefully isolated with cotton rolls. A saliva ejector was used to avoid salivary contamination of the samples. Two paper strips (Periopaper, Amityville, NY, USA) were consecutively inserted into the crevice at the mesial and distal midpoints until mild resistance was felt. The strips were left *in situ* for 30 s and then transferred, for volume determination, to the chair-side located Periotron 8000 (Oraflow Inc., Plainview., NY, USA) which was calibrated using known volumes of phosphate-buffered saline (PBS). Two strips were immediately placed in a labeled tube containing 700 µl PBS and transported to the laboratory. Following 15 min agitation at room temperature, the strips were removed and the eluates centrifuged for 5 min at 3000 *g* to remove plaque and cellular elements. The samples were stored at −80 °C for subsequent assays.

Enzyme assay

NE was determined in ELISA plates by measurement of *p*-nitroanilide resulting from hydrolysis of the neutrophil elastase-specific peptide, *N*-methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide. Hydrolysis of this substrate is accompanied by a color change that can be quantified using an immunoassay plate reader. This method measures functionally active NE levels. Standard solutions of NE (range 2–1000 µU/µl) were prepared with ELISA

diluent buffer. On each microtiter plate, 25 µl of PBS and 200 µl of substrate were added to two wells in the first column as the substrate blank. Duplicate 25 µl samples of each standard and 200 µl of substrate were placed in standard wells. A total of 25 µl of sample and 200 µl of substrate were added to sample wells. Plates were incubated for 2.5 h at 37 °C. Color change was determined by an enzyme immunoassay plate reader with a 405-nm filter. Crevicular fluid NE levels were calculated from the standard curve and defined as µU/µl for concentration and as µU/site for total enzyme activity.

Cytokine assay

Levels of IL-1β and TNF-α in samples were determined by using an appropriate commercial ELISA kit (Immunotech, Marseilles, France); 100 µl of eluted sample was assayed according to the kit's instructions. The results were read using a microplate reader at 405 nm wavelength. Concentrations of the cytokines in each 100 µl sample were determined by generation of a standard curve for comparison. Concentrations of cytokines were corrected for PICF volume and were defined as pg/µl. Total amounts of cytokines were expressed as pg/site.

Statistical analysis

Statistical comparisons between enzyme activity, cytokine levels and clinical parameters were made on an implant basis using pooled patient data. Because implant site data are affected both by local factors and overall patient influences (Sterne et al. 1990), weighted implant site parameters were calculated for statistical comparisons. For this purpose, average implant values were first obtained as the mean of mesial and distal measurements. Mean patient parameters were obtained from these values and then weighted site data calculated by adding the mean patient value to site values and dividing the sum by 2.

The implants were divided into two groups according to smoking habits of the patients, implants of nonsmokers group (INSM) ($n = 24$) and implants of smokers group (ISM) ($n = 18$). The INSM group was also divided into subgroups according to gingival inflammation (GI score > 1 (GI > 1) and GI score > 1 (GI > 1)) and pocket depth measurements (shallow pockets PD ≤ 3 mm and deep pockets PD > 3 mm). The significance of the difference between groups or subgroups was investigated by Mann-Witney *U*-test. The relationships among PICF cytokine levels and NE activity and clinical parameters were analyzed by using Spearman's rank correlation test.

Table 1. **Correlations between IL-1β, TNF-α, NE levels in PICF and clinical parameters (Spearman rank correlation coefficients, whole implants)**

	n	PD	PI	GI	PICFvol
Total					
IL-1β	42	0.104	− 0.272	− 0.097	0.094
TNF-α	42	0.078	0.406**	0.485**	0.333*
NE	42	0.090	− 0.229	− 0.177	− 0.219
Concentration					
IL-1β	42	− 0.030	− 0.387*	− 0.337*	− 0.354*
TNF-α	42	− 0.044	0.281	0.222	− 0.104
NE	42	− 0.070	− 0.355*	− 0.306*	− 0.417**

*$P < 0.05$. **$P < 0.01$.

Table 2. **Correlations between IL-1β, TNF-α, NE levels in PICF and clinical parameters (Spearman rank correlation coefficients, INSM group)**

	n	PD	PI	GI	PICFvol
Total					
IL-1β	24	0.396	0.267	0.602**	0.710**
TNF-α	24	− 0.198	0.205	0.191	0.044
NE	24	0.464*	0.306	0.607**	0.151
Concentration					
IL-1β	24	0.221	0.330	0.473*	0.245
TNF-α	24	− 0.302	0.097	− 0.051	− 0.345
NE	24	0.445*	0.236	0.604**	− 0.056

*$P < 0.05$. **$P < 0.01$.

Table 3. **Comparisons between GI ≤ 1 and GI > 1 groups regarding clinical parameters and PICF constituents**

	GI ≤ 1 (*n* = 13) Mean (SD)	GI > 1(*n* = 11) Mean (SD)	*P*
Clinical parameters			
PD (mm)	3.06 (0.53)	3.56 (0.46)	0.017*
PI	0.49 (0.87)	0.83 (0.18)	0.071
GI	0.77 (0.39)	1.91 (0.20)	0.000*
PICF (µl)	0.47 (0.17)	0.66 (0.15)	0.006*
Cytokine and enzyme levels			
Total			
IL-1β (pg/site)	33.80 (17.77)	70.24 (23.35)	0.001*
TNF-α (pg/site)	13.90 (12.18)	18.80 (6.80)	0.077
NE (µU/site)	820.10 (417.22)	2476.40 (1448.21)	0.008*
Concentration			
IL-1β?(pg/µl)	74.36 (34.50)	105.10 (22.16)	0.022*
TNF-α (pg/µl)	40.16 (51.89)	31.41 (11.47)	0.622
NE (µU/µl)	2232.74 (1299.13)	4480.34 (2490.99)	0.026*

Statistically significant, Mann–Whitney *U*-test.

Results

A total of 42 osseointegrated root-form implants were evaluated and sampled in the study. Implants had been in place for a mean of 86.57 months (range 12–108 months) and had been observed to be primarily osseointegrated before loading. None of the implants had excessive radiographic bone loss (mean 17.26%, range 0–30%), mobility or fistulae. Implants were in function and accepted as being clinically satisfactory. Of the 14 patients participated in the study, 10 were nonsmokers (24 implants) and four were smokers (18 implants). IL1-β and NE were detected in PICF of all the implants sampled, whereas TNF-α was recovered from 37 of 42 implant samples.

During the statistical analysis of whole implant data including implants of smoker patients, we observed random negative and positive correlations between clinical parameters and PICF cytokine levels and NE activity (Table 1). These unexpected results led us to treat data separately for the implants of nonsmoker patients. Significant positive correlations between clinical parameters and PICF IL-1β levels and NE activity were then found (Table 2). NE showed significant positive correlations with clinical parameters PD and GI ($P<0.05$). IL-1β exhibited positive significant correlations with GI and PICF volume ($P<0.05$). However, correlations were stronger when the data were expressed as total amounts or as total activity rather than concentration.

The mean data for clinical parameters, PICF cytokine levels and NE activity of the GI ≤ 1 group vs. GI > 1 group are presented in Table 3. As noted, PD and PICF volume were significantly greater in GI > 1 group than GI ≤ 1 group ($P<0.05$). The GI ≤ 1 group presented significantly lower IL-1β levels and NE activity than the GI > 1 group ($P<0.05$). The results were similar for PICF levels of IL-1β and NE activity when the data were expressed as total amounts or concentrations. Table 4 presents the mean data for clinical parameters, PICF cytokine levels and NE activity of the PD ≤ 3 mm group vs. PD > 3 mm group. The differences between groups were not significant for clinical parameters ($P>0.05$). Among the PICF constituents, only the mean total NE activity was significantly higher in the PD > 3 mm group ($P<0.05$).

The mean data for clinical parameters, PICF cytokine levels and NE activity and the results of comparisons between the INSM group and ISM group are given in Table 5. All the clinical parameters in the INSM group were significantly lower than in the ISM group ($P<0.05$). When the mean total amounts of cytokines and total NE activity levels were compared, significantly decreased NE levels and significantly increased TNF-α levels were observed in the ISM group ($P<0.05$). Decreased total amount of IL-1β was close to significance ($P=0.053$), and was significant when the data were expressed as concentration ($P>0.05$).

Table 4. **Comparisons between PD ≤ 3 mm and PD > 3 mm groups regarding clinical parameters and PICF constituents**

	PD ≤ 3 mm (n = 15) Mean (SD)	PD > 3 mm (n = 9) Mean (SD)	P
Clinical parameters			
PD (mm)	2.80 (0.32)	4.11 (0.55)	0.000*
PI	0.72 (0.77)	0.53 (0.43)	0.370
GI	1.14 (0.57)	1.55 (0.64)	0.091
PICF (μl)	0.53 (0.19)	0.62 (0.16)	0.144
Cytokine and enzyme levels			
Total			
IL-1β (pg/site)	43.08 (22.99)	62.88 (30.72)	0.114
TNF-α (pg/site)	17.92 (10.85)	13.18 (8.73)	0.387
NE (μU/site)	1125.05 (974.28)	2336.22 (1494.44)	0.040*
Concentration			
IL-1β (pg/μl)	81.18 (34.55)	100.56 (27.37)	0.180
TNF-α (pg/μl)	42.78 (46.64)	25.09 (15.26)	0.421
NE (μU/μl)	2594.60 (1708.29)	4376.69 (2590.45)	0.089

Statistically significant, Mann–Whitney *U*-test.

Table 5. **Comparisons between INSM and ISM groups regarding clinical parameters and PICF constituents**

	INSM Mean SD (n = 24)	ISM Mean SD (n = 18)	P
Clinical parameters			
PD (mm)	3.29 (0.55)	3.97 (0.69)	0.001*
PI	0.65 (0.66)	1.69 (0.93)	0.001*
GI	1.29 (0.61)	1.72 (0.44)	0.001*
PICFvol	0.56 (0.18)	0.74 (0.27)	0.037*
Cytokine and enzyme levels			
Total			
IL-1β (pg/site)	50.50 (27.31)	36.55 (18.26)	0.053
TNF-α (pg/site)	16.14 (10.18)	23.18 (11.22)	0.045*
NE (μU/site)	1579.24 (1308.96)	580.77 (511.63)	0.003*
Concentration			
IL-1β (pg/μl)	88.45 (32.85)	65.49 (44.23)	0.016*
TNF-α (pg/μl)	36.15 (38.49)	43.30 (27.33)	0.121
NE (μU/μl)	3262.88 (2210.68)	1000.71 (701.63)	0.000*

Statistically significant, Mann–Whitney *U*-test.

Discussion

The results of the present study showed that implants with inflamed gingiva have greater IL-1β levels and NE activity compared to implants with slightly or noninflamed gingiva. Moreover, we found that IL-1β levels and NE activity in PICF significantly correlated with peri-implant gingival inflammation. Cross–sectional associations have been reported between elevated activities of proteolytic enzymes in PICF and inflammatory signs of peri-implant tissues (Apse et al. 1989). Our find-

ings are in general agreement with previous studies reporting correlations between gingival inflammation and increasing amounts of IL-1β and NE in GCF or PICF (Eley et al. 1991; Cox & Eley 1992; Hou et al. 1995; Rawlinson et al. 2000). Additionally, our results support studies that have showed IL-1 can significantly enhance elastase release by activated neutrophils (Owen et al. 1997; Brandolini et al. 1996).

In the present study, PICF cytokine levels and enzyme activity were expressed both as total amount and as concentration. Positive correlations between IL-1β or NE and the clinical parameters are more marked with total amount of cytokines or total enzyme activity. Eley et al. (1991) have claimed that the correlations between clinical parameters and PICF elastase levels were better for total enzyme activity than concentration. In a previous study, where myeloperoxidase and elastase levels of GCF were presented both as total activity or as concentration, it has been reported that data presentation by use of total activity was more sensitive in the reflection of the existing clinical periodontal status (Yamalik et al. 2000). Our results agree with findings of these studies (Eley et al. 1991; Yamalik et al. 2000). PICF levels of IL-1β and NE appear more closely associated with clinical implant health status when these PICF constituents are reported in total amounts per site rather than as concentrations.

In the present study, it was interesting to note that implants with deep pockets (PD > 3 mm) did not have more gingival inflammation or more plaque than the implants with shallow pockets (PD ≤ 3 mm). Among PICF constituents studied, only NE is able to discriminate shallow and deep peri-implant pockets. Probing pocket depth and probing attachment levels are reliable criteria for periodontal health status (Goodson 1986; Lang & Brägger 1991). However, there is some controversy in the literature regarding the significance of measuring probing depths around dental implants. It has been reported that probing depths are consistently greater around stable implants when compared to natural teeth (Akagawa et al. 1989; Apse et al. 1989). The altered probing depths of implants may be directly related to bone loss, whereas changes around teeth with attached dentogingival fibers may rather be related to attachment loss and changes in inflammation (Schou et al. 1993). In failing implants, progressive deepening of pockets is always found, but absolute pocket depth is not always indicative of implant failure (Rams & Link 1983; Rams et al. 1984). Probing pocket depths appear to have same specifity, but low sensitivity, for determining disease around implants when compared to teeth. The diagnostic applicability of probing pocket depth around implants remains to be determined.

The findings of the present study revealed that implants of smoker patients had significantly decreased NE activity and IL-1β levels and significantly increased TNF-α levels in PICF. Furthermore, we determined deeper pockets, higher PI and GI scores and greater PICF volume in smokers. Decreased NE levels (Alavi et al. 1995) and increased TNF-α levels in GCF (Boström et al. 1998, 1999) have been previously demonstrated in smoker periodontitis patients. Pauletto et al. (2000) have claimed that the low oral elastase levels of smokers should be interpreted as an indication of an abnormal leukocyte function and as increased risk for periodontitis. The limited number of smoker patients is the drawback in this study. Nevertheless, it is likely that in implant patients who smoke, neutrophils, rather than migrating to the peri-implant sulcus, are accumulated in peri-implant tissue where they release their constituents, leading to increased degradation of connective tissue components. However, this hypothesis needs to be confirmed in further studies with greater numbers of smoker implant patients.

TNF-α is a proinflammatory cytokine that possesses similar activities to IL-1β. In an earlier study, Panagakos et al. (1996) was not able to detect TNF-α in the crevicular fluid of implants. To our knowledge, this is the first study demonstrating the presence of TNF-α in PICF of implants. In comparison, TNF-α levels in PICF were somewhat lower than IL-1β levels and were not correlated with clinical parameters. Based on the findings of the present study, it remains to be established whether TNF-α level in PICF has a value as a measure of peri-implant health status.

The importance of regular maintenance recall visits is generally accepted in the field of oral implantology (Wilson 1991; Brägger et al. 1994). There is a need for simple and reliable clinical tests to detect peri-implant pathology at an early and reversible stage. The main aim of the present study was to explore whether proinflammatory mediators IL-1β and TNF-α or a serine protease NE in PICF provide early diagnostic or prognostic information as to the status of the implant. This cross-sectional study was conducted on a implant patient population exhibiting clinical signs of peri-implant mucositis and peri-implantitis. But all the implants sampled were in function and accepted as clinically satisfying. Among the PICF constituents studied, NE and IL-1β appear to reflect clinical health status of implants of patients who do not smoke. In future studies, it will be necessary to relate PICF NE activity and IL-1β levels to progressive peri-implantitis in a full longitudinal study. However, IL-1β levels and NE activity in PICF of smoker patients should be interpreted cautiously.

Résumé

Le but de cette etude a été de determiner les teneurs en interleukines-1β (IL1β) et en facteurs alpha de la néecrose tumorale (TNF-alpha) ainsi que l'activité de élastase neutrophile (NE) dans le fluide créviculaire paroimplantaire (PICF) chez des fumeurs et des non-fumeurs, et d'examiner leaurs relations avec des parameters cliniques. Quarante-deux implants dentaires en forme de raçine chez quartorze patienrts ont été examines pour l'indice de plaque modifié (PII), l'indice gingival modifié (GI) et la profondeur au sondage (PD). Les habitudes tabagiques des patients ont été enregistrées. Le PICF des implants a été prélevé à l'aide de bandelettes de papier Perio et les niveaux de IL-1β et de TNF-alpha ont eté déterminés par ELISA. NE a été analysé avec un substrat chromosonmique spécifique du neutrophile, le N-méthoxysuccinyl-Ala-Ala-Pro-Val-p-nitroanilide. Les niveaux de cytokine et d'enzymes dans le PICF ont été exprimés en quantitité totale et en concentration. L'activité NE dans le PICF était en corrélation significative avec GI et PD, et les teneurs IL-1β avec GI et le volume de PICF (p>0.05). L'activité totale NE dans les implants avec poches profondes (>3 mm) était plus importante qu'au niveau des implants avec poches peu profondes (>3 mm) (p>0.05). Les implants chez les furneurs avaient significativement des teneurs en TNF-alpha supérieures par rapport aux implants des non-fumeurs (p>0.05). Les découvertes de l'étude présente indiquent que l'activité NE et les teneurs en IL-1β dans PICF peuvent êetre utilisées pour mesurer l'état de santé de l'implant chez les non-fumeurs.

Zusammenfassung

Das Ziel dieser Studie war die Bestimmung der Interleukin-1beta (IL-1beta) und Tumornekrosefaktor-alpha (TNF-alpha) Niveaus und der Aktivität der neutrophilen Elastase (NE) in der peri-implantären Sulkusflüssigkeit (PICF) von Rauchern und Nicht-Rauchern. Die Beziehung zwischen den obengenannten Faktoren und den klinischen Parametern sollte untersucht werden. Insgesamt wurden 42 enos-

sale wurzelförmige Implantate von 14 Patienten klinisch mittels modifiziertem Plaqueindex (PI), modifiziertem Gingivalindex (GI) und Sondierungstiefen (PD) untersucht. Die Rauchgewohneiten der Patienten wurden aufgezeichnet. PICF der Implantate wurde mittels Periopapierstreifen gesammelt. Die IL-1beta und TNF-alpha Niveaus wurden mittels enzymgebundenem Immunabsorbtionstest (ELISA) bestimmt. NE wurde mit einem neutrophilspezifischen Substrat, N-methoxysuccinyl-Ala-Ala-Pro-Val-p-nitroanilid, analysiert. Die Niveaus der Zytokine und der Enzyme im PICF wurden als totale Menge/Aktivität und als Konzentrationen ausgedrückt. Die NE Aktivität im PICF korrelierte mit dem GI und der PD, und die IL-1beta Niveaus korrelierten mit dem GI und dem PICF Volumen ($p<0.05$). Die Korrelationen waren besser, wenn die PICF Niveaus als totale Menge IL-1beta und als totale NE Aktivität ausgedrückt wurden. Die Implantate mit entzündeter Gingiva (GI>1) zeigten höhere Niveaus an IL-1beta und NE Aktivität als Implantate mit nichtentzündeter oder leicht entzündeter Gingiva (GI≤) ($p<0.05$). Die totale NE Aktivität war bei implantaten mit tiefen Taschen (PD≥3mm) grösser als bei Implantaten mit seichten Taschen (PD≤3mm) ($p<0.05$). Die Implantate der Raucher zeigten eine signifikant tiefere PICF NE Aktivität und tiefere IL-1beta Niveaus und signifikant höhere TNF-alpha Niveaus als die Implantate der Nicht-Raucher ($p<0.05$). Die Ergebnisse der vorliegenden Studie zeigen, dass NE Aktivität und IL-1beta Niveaus im PICF verwendet werden könnten, um den Gesundheitsstatus der Implantate bei Nicht-Rauchern zu bestimmen.

Resumen

La intención de este estudio fue determinar los niveles de interleuquina-1β (IL-1β), de factor-α de necrosis tumoral (TNF-α) y la actividad de la elastasa neutrófila (NE) en el fluido periimplantario (PICF) de pacientes fumadores y no fumadores, e investigar sus relaciones con los parámetros clínicos. Se examinaron clínicamente un total de 42 implantes dentales endoóseos con forma de raíz de 14 pacientes, por medio de índice de placa (PI), índice de placa modificado (GI) profundidad de sondaje (PD). Se recogieron los hábitos de tabaquismo de los pacientes. Se tomaron los PICF de los implantes con tiras de Periopaper y se determinaron los niveles de IL-1β y de TNF-α por medio de prueba de inmunoabsorción ligado a enzimas (ELISA). Se analizó la NE con un sustrato cromogénico neutrofílico específico, N-methoxysuccinyl-Ala-Ala-Pro-Val-p-nitroanilida. Los niveles de citoquina y de enzima en el PICF se expresaron como cantidad/actividad total y como concentraciones. La actividad de NE en el PICF se correlacionó significativamente con el GI y el PD, y los niveles de IL-1β con el volumen del GI y de PICF ($p<0.05$). Las correlaciones fueron mayores cuando los niveles de PICF se expresaron como el total de la cantidad de IL-1β y como el total de la actividad NE. Los implantes con la encía inflamada (GI>1) tuvieron niveles mayores de IL-1β y actividad NE que los implantes con encía ligeramente inflamada o sin inflamación (GI≤1) ($p<0.05$). La actividad total de NE en implantes con bolsas profundas (PD>3mm) fue mayor que en implantes con bolsas poco profundas (PD≤3mm) ($p<0.05$). Los implantes de los pacientes fumadores tuvieron una significativamente menor actividad NE en PICF y niveles IL-1β, y niveles significativamente mas altos de TNF-α que los implantes de los no fumadores ($p<0.05$). Los hallazgos del presente estudio indican que la actividad NE y los niveles IL-1β en PICF pueden usarse para medir el estado de salud del implante de pacientes que no fuman.

要旨

本研究は、喫煙患者及び非喫煙患者のインプラント周囲歯肉溝液（PICF）においてインターロイキン１β（IL-1β）と腫瘍壊死因子－α（TNF－α）の濃度と好中球エラスターゼ（NE）活性を測定し、その臨床的パラメータとの相関性を調べることを目的に行った。

患者１４名の合計４２本の骨内ルートフォーム・インプラントをプラーク・インデックス（PI）変法、歯肉インデックス変法（GI）及びプローピング深さ（PD）によって臨床的に評価した。患者の喫煙癖を記録した。インプラントのPICFは、ペリオペーパー・ストリップにより採取し、IL-1β及びTNF－αの濃度を酵素免疫測定法（ELISA）により測定した。NEは好中球特異的色素生成性基質 N-methoxysuccinyl-Ala-Ala-Pro-Val-p-nitroanilide によって解析した。PCIF中のサイトカインと酵素の濃度は、総量/活性及び濃度として表現した。PICF中のNE活性はGI及びPDと、またIL-1β濃度はGIとPICFの量と有意に相関していた（p＜0．05）。PICF中の濃度をIL-1β総量及びNEの総活性として表現した場合、相関性は強くなった。炎症歯肉を伴っているインプラント（GI＞1）は、炎症がない、あるいはわずかである歯肉（GI≦1）を伴うインプラントよりも、IL-1β濃度とNEの活性が、高かった。ポケットが深い（PD＞3mm）インプラントのNE総活性は、浅いポケット（PD≦3mm）のインプラントよりも高かった（p＜0．05）。喫煙患者のインプラントは、非喫煙患者に比べ、PICFのNE活性とIL-1β濃度は有意に低く、TNF－αの濃度は有意に高かった（p＜0．05）。本研究の所見は、PICF中のNE活性とIL-1β濃度は、喫煙しない患者におけるインプラントの健全度の測定に有効でありうることを示唆している。

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