Correlations Between Clinical Parameters and Interleukin-6 and Interleukin-10 Levels in Saliva from Totally Edentulous Patients with Peri-implant Disease

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Purpose: *The purpose of the present study was (1) to assess the relationship between clinical parameters and concentrations of the proinflammatory cytokine IL-6 and the anti-inflammatory cytokine IL-10 in the saliva of totally edentulous patients with implant-supported overdentures; (2) to assess whether estimation of IL-6 and IL-10 levels in saliva could be a useful laboratory tool to detect changes preceding serious clinical complications.* ***Materials and Methods:*** *Thirty healthy adult volunteers (14 men and 16 women) with implant-supported overdentures were recruited from Tallinn Dental Clinic. The biochemical and clinical parameters evaluated were the levels of IL-6 and IL-10 in saliva, pocket probing depth (mm), Gingival Index (0,1, 2, or 3), and bleeding on probing (0 or 1).* ***Results:*** *The level of IL-6 in saliva in the peri-implant disease group was significantly elevated compared to the healthy group. IL-10 could be detected only in the saliva of patients with peri-implant disease, and it did not appear at detectable amounts in saliva of healthy controls. In addition, the levels of IL-6 and IL-10 in peri-implant disease group were positively correlated with clinical parameters.* ***Conclusions:*** *These data suggest that a significant relationship exists between the amount of a proinflammatory cytokine (IL-6) and the inflammatory response in peri-implant tissue. The results also suggest that IL-6 and IL-10 could be used as markers of peri-implant disease together and that the level of the latter cytokine gives additional information about the potency of an organism's integrated immune response for maintenance of inflammatory balance.* (More than 50 references) Int J Oral Maxillofac Implants 2006;21:543–550

Key words: *dental implants, interleukin-6, interleukin-10, peri-implant disease, peri-implantitis, saliva*

The long-term success of osseointegrated implants as replacements for missing teeth relies, among other factors, on maintaining the integrity of the biological seal of the peri-implant tissues.[1] The peri-implant sulcus is, anatomically, functionally, and environmentally quite similar to the periodontal crevice[2,3] and provides a niche for the colonization and growth of oral microorganisms. When the host response to plaque accumulation results in reversible soft tissue reactions, the resultant condition is termed *peri-implant mucositis,* whereas the term *peri-implantitis* is used to describe irreversible inflammatory reactions with the loss of supporting bone in the tissues surrounding a functioning implant.[4] Animal and human studies have shown that peri-implant infection appears to have similar clinical, radiographic, and histologic features to periodontitis.[5–8]

Recent evidence indicates that inflammatory cytokines released by the host's cells in response to bacterial products such as lipopolysaccharide and endotoxins are substantially responsible for the breakdown processes of the periodontium in periodontitis.[9,10]

An important aspect of the host response is cell-mediated immunity, of which T cells are the main components. The majority of T cells express the T cell

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receptor that recognizes processed antigens in conjunction with major histocompatibility complex molecules. These cells release a variety of cytokines following antigen recognition. Two subsets of CD4+ T helper (Th) cells have been defined based on the pattern of cytokine secretion. Cells of the Th1 subset secrete interleukin (IL)-2, interferon (INF)-γ, and INF-α, while the Th2 cell subset secretes IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13.[11] The synthesis of IL-2, IL-6, IL-10, and IL-13 is not tightly restricted to a single subset.[11]

Although cytokines are produced by locally infiltrated immunocompetent cells such as T cells and monocytes at disease sites, cell types that normally compose the periodontal tissue—such as fibroblasts, epithelial cells, and endothelial cells—are also involved in cytokine production during the inflammatory response.[11]

An inflammatory cytokine is defined as a cytokine that is induced during the course of an inflammatory response and is closely associated with its onset and/or progression. IL-1α, IL-1β, IL-6, IL-8, and TNF-α are generally classified as inflammatory cytokines.[11] Since one prominent feature of periodontal disease is resorption of alveolar bone, particular attention has been paid to the roles of IL-1α, IL-1β, IL-6, IL-8, and TNF-α in the pathogenesis process because of their enhancement of bone resorption.[11]

Some studies[12,13] have shown that IL-1β is present at elevated levels in the gingival crevicular fluid (GCF) and in tissue from periodontal pockets. This cytokine stimulates bone resorption, prostaglandin synthesis, and protease production by many cell types, including fibroblasts and osteoblasts.[14–18] IL-6 is a proinflammatory cytokine and central mediator of the acute-phase response. This pleiotropic cytokine stimulates B cell differentiation and T cell activation as well as hepatocyte production of acute phase proteins, including C-reactive protein (CRP).[19] In addition, it can induce bone resorption by activating cells to release secondary mediators that are responsible for tissue destruction.[20,21] Spontaneous production of IL-6 has been reported in mononuclear cells isolated from inflamed gingival tissues of patients with periodontitis.[22] The presence of IL-6–bearing cells in inflamed gingival tissue also has been demonstrated by other researchers.[23,24] In a longitudinal study, IL-6 content in GCF was found to be correlated with the severity of periodontal disease.[25]

IL-10, a cytokine with potent anti-inflammatory properties, has been implicated in the regulation of both cellular and humoral immune responses.[26–29] Studies indicate that lack of IL-10 can render a host susceptible to a bacteria-initiated chronic inflammatory condition.[30] IL-10 has a major role in regulating proinflammatory cytokine levels in vivo. For example, IL-1 and tumor necrosis factor (TNF) production in response to various inflammatory stimuli are elevated in the absence of IL-10 and can be curtailed by IL-10 administration.[28,31,32] Also, IL-10 has been reported to downregulate the production of both IL-1[33–37] and IL-6.[38,39] Sasaki and associates[40] found that IL-10 suppresses infection-stimulated bone resorption in vivo.

Although extensive research has been done in the area of periodontal inflammatory mediators, few have studied the role of the host immune response in peri-implant disease. To the authors' knowledge no studies have been conducted regarding the roles of IL-6 and IL-10 in the pathogenesis of peri-implant disease.

Recent advances in the understanding of biologic events involved in the pathogenesis of periodontitis indicate that proinflammatory cytokine IL-6 may also be operative in the pathogenesis of peri-implant disease. On the contrary, anti-inflammatory cytokine IL-10 may suppress peri-implant disease associated with tissue destruction.

It was decided to study edentulous patients with peri-implant disease. The patients' dentition consisted of full maxillary dentures and implant-supported mandibular overdentures. Because the participants had no natural teeth, whole saliva was chosen as a medium for diagnostics. The overall advantages of using saliva as a diagnostic fluid were recently reviewed.[41]

Saliva has the advantage of being simple and noninvasive to collect in comparison to peri-implant sulcus fluid. Both issues were important because of the age of patients (range, 62 to 70 years). Quantification of cytokines in saliva has been performed for patients with several pathologic conditions: in denture stomatitis,[42] HIV-positive smokers with oropharyngeal candidiasis,[43] and oral lichen planus.[44]

The purposes of this study were *(1)* to determine the levels of IL-6 and IL-10 in the saliva of patients with peri-implant disease and the correlation between these levels and clinical parameters and *(2)* to assess whether estimation of IL-6 and IL-10 levels in saliva could be a useful laboratory tool to detect changes preceding serious clinical complications.

MATERIALS AND METHODS

Patient Population

Thirty totally edentulous patients (14 men, 16 women) with implant-supported overdentures (Ankylos dental implants; Dentsply Friadent, Mannheim, Germany) who were receiving maintenance care participated in this study. All the patients had mandibular overdentures supported by 2 implants. The mean time in function was 67.2 ± 3.9 months (range, 61 to 72 months).

Prior to the start of the study the subjects gave their informed consent. Inclusion criteria were *(1)* the presence of 2 endosseous dental implants, *(2)* appropriate overdentures, *(3)* the same soft tissue biotype among all the patients, *(4)* healthy oral mucosa (eg, no denture stomatitis), *(5)* no history of antibiotic treatment prior to the study for 3 months, *(6)* negative history of systemic diseases affecting cytokine levels in saliva, *(7)* no history of medical conditions that required antibiotic prophylaxis, *(8)* no history of chronic corticosteroid use, and *(9)* no history of medication interfering with saliva secretion. A complete oral examination was given prior to sialometry and measurement of clinical parameters.

Table 1 Clinical Assessment and Diagnosis of Implants

Diagnosis	PPD	GI	BOP
Patients with peri-implant disease (n = 12)	≥ 4 mm	≥ 1	1
Healthy patients (n = 18)	≤ 3 mm	0	0

PPD = pocket probing depth; GI = Gingival Index; BOP = bleeding on probing.

Clinical Examination

The clinical evaluations were performed by a single examiner (SL). The clinical examinations included assessment of peri-implant pocket probing depth (PPD), Gingival Index[45] (GI) (0, 1, 2, or 3), and bleeding on probing (BOP) (0 or 1). Clinical measurements were taken at 4 sites (mesial, buccal, distal, and lingual). The PPD was measured to the nearest millimeter with a pressure-calibrated periodontal probe with a tip diameter of 0.5 mm and a probing force of 0.25 N (Click-Probe; Hawe-Neos Dental, Bioggio, Switzerland).[46,47]

Criteria for Health and Disease

Studied patients were categorized on the basis of PPD, GI, and BOP as either patients with peri-implant disease (peri-implantitis and peri-implant mucositis) or healthy patients. Briefly, *peri-implantitis* is defined as inflammation around implants with loss of supporting bone, and *peri-implant mucositis* is defined as reversible inflammation of the soft tissues with no loss of supporting bone. To categorize the patients, the clinical parameters are shown in Table 1. The criteria have been described in previous reports.[48,49] If 1 of the implants was healthy and the other showed signs of peri-implant disease, a patient was categorized as diseased.

Saliva Collection

Whole unstimulated (resting) saliva specimens were obtained in the morning, before clinical evaluation. No oral stimulation was permitted for 90 minutes prior to collection. Five milliliters of whole saliva was collected by standardized gentle suction from the floor of the mouth into a sterile centrifuge tube. The saliva was centrifuged for 5 minutes at 800 *g,* separated into 0.5-mL aliquots, and frozen at –70°C until use.

All samples were assayed for blood contamination using an enzyme immunoassay kit for transferrin (Salimetrics, State College, PA). Transferrin is a large protein prevalent in blood at very high concentrations. Transferrin's large size prevents it from being passively or actively transported from the general circulation into saliva. Saliva samples were assayed for transferrin using an enzyme immunoassay as described by Kivlighan and colleagues.[50] Contaminated samples were not included in the study.

The total protein content was determined by the method of Lowry and coworkers[51] using serum albumin as a standard. Saliva from peri-implant disease group had a protein concentration of 2.91 ± 0.63 mg/mL; saliva from the healthy control group had a protein concentration of 2.38 ± 0.51 mg/mL.

Cytokine Assay

The amounts of IL-6 and IL-10 in the saliva samples were determined using commercially available enzyme-linked immunoadsorbent assays (ELISAs) (R&D Systems, Inc., Minneapolis, MN) specific to each cytokine. The assays were sandwiched and performed according to the manufacturer's instructions using human recombinant standards. All samples were tested in duplicate. The IL-6 and IL-10 contents were expressed as pg/mg protein. Previous studies have confirmed that cytokines are stable in saliva.[52]

Statistical Analysis

For categorical variables, number and percentage of patients in different categories are presented; for continuous variables, mean and standard deviation (SD) are presented. For statistical comparison between groups, Fisher's exact test was used for categorical variables and Wilcoxon Mann-Whitney test for continuous variables. All tests were conducted at the conventional 5% significance level. For IL-6 and IL-10 levels geometric means with 95% confidence intervals were calculated in subgroups defined by PPD, BOP, and GI. These results are presented in the form of error bar graphs (Figs 1 to 3). For statistical analysis and graphs, the software package R 2.0.0 for Windows (http://www.r-project.org) was used.

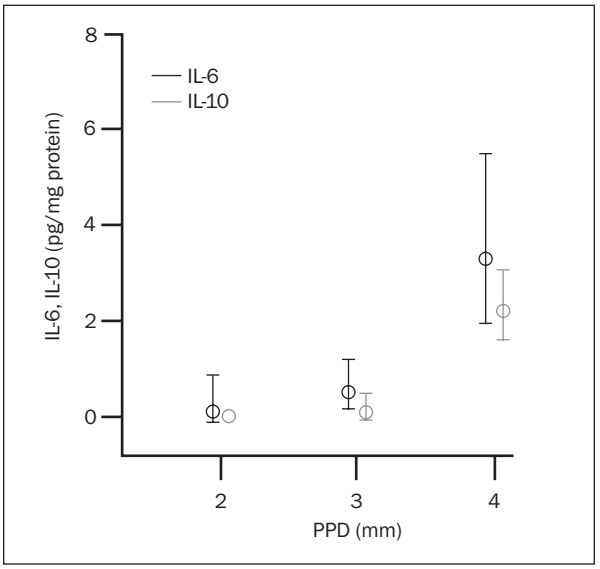

Fig 1 Geometric mean (95% CI) of total amounts of IL-6 and IL-10 in saliva in healthy and diseased patients in association with PPD.

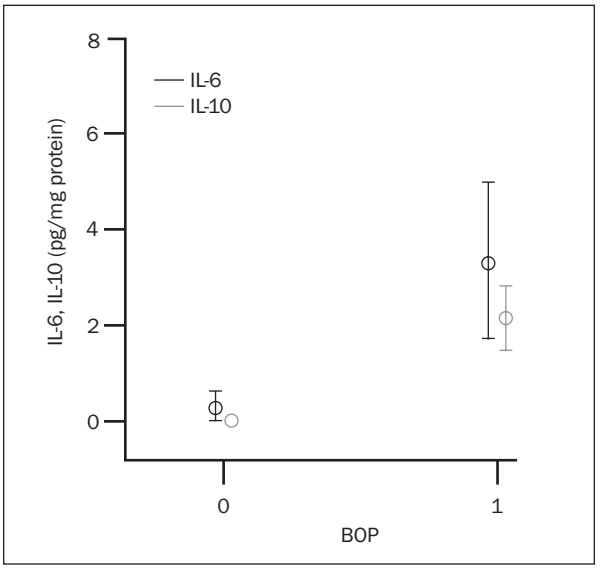

Fig 2 Geometric mean (95% CI) of total amounts of IL-6 and IL-10 in saliva in healthy and diseased patients in association with BOP.

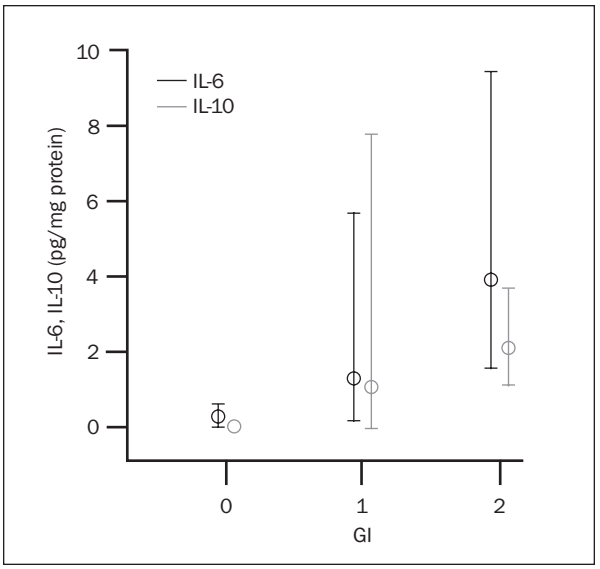

Fig 3 Geometric mean (95% CI) of total amounts of IL-6 and IL-10 in saliva in healthy and diseased patients in association with GI.

RESULTS

Patient Data and Clinical Results

Twelve patients showed signs of peri-implant disease. The 18 patients with healthy peri-implant tissues were used as a control group.

Table 2 shows clinical status of each group including PPD, GI, and BOP. Statistically significant differences for 2 groups were observed for all clinical parameters.

Biochemical Analysis of Saliva

Table 3 shows the total amounts of IL-6 and IL-10 in saliva in healthy and diseased groups. Significant differences were found between the 2 groups in levels of both IL-6 and IL-10 ($P = .001$ and $P < .001$, respectively).

Clinical Parameters and Levels of Cytokines

Figure 1 shows the relationship between PPD and cytokine concentration. Concentration of IL-6 and IL-10 increased as PPD increased. Figures 2 and 3 demonstrate the relationship between BOP, GI, and cytokine concentration. Activity of IL-6 and IL-10 was higher if BOP was present, and cytokine activity increased with GI score.

Table 4 shows the distribution of IL-6 and IL-10 levels using different levels of clinical parameters. As can be seen, in all patients with PPD greater than 3 mm, the levels of IL-6 and IL-10 were higher than 1.5 pg/mg protein and 1.0 pg/mg protein, respectively. In patients with a GI score of 0, IL-10 could not be detected, whereas all the patients with a GI score of at least 2 had IL-6 and IL-10 levels exceeding 1.5 pg/mg protein and 1.0 pg/mg protein, respectively. In patients with no bleeding present, IL-10 could not be detected. All the patients with bleeding on probing had IL-6 and IL-10 levels exceeding 1.5 pg/mg protein and 1.0 pg/mg protein, respectively.

DISCUSSION

The present study showed a significant difference in the levels of IL-6 and IL-10 in saliva between diseased

Table 2 Clinical Data from Healthy Patients and Patients with Peri-implant Disease

	PPD (mm)*	GI > 1†	BOP = 1†
Healthy (n = 18)	2.63 (0.52)	0	0
Diseased (n = 12)	3.85 (0.38)	57	100
P	.003	.026	.0002

*Mean (SD).
†Percentage of patients shown.

Table 3 Total Amounts of IL-6 and IL-10 in Saliva of Healthy Patients and Patients with Peri-implant Disease

	IL-6 (pg/mg protein)*	IL-10 (pg/mg protein)*
Healthy (n = 18)	0.30 (0.37)	0.00 (0.00)
Diseased (n = 12)	3.33 (1.77)	2.14 (0.74)
P	.001	< .001

*Mean (SD).

Table 4 Distribution of IL-6 and IL-10 Levels Using Different Levels of PPD, Different GI Scores, and Different BOP Values

	PPD			GI			BOP	
	< 3 mm	3 mm	≥ 4 mm	0	1	≥ 2	0	1
IL-6 levels (pg/mg protein)*	0.11 (0.18)	0.62 (0.63)	3.61 (1.76)	0.31 (0.40)	1.61 (0.96)	4.27 (1.88)	0.30 (0.37)	3.33 (1.77)
% of patients with IL-6 levels > 1.5 pg/mg protein	0	17	100	0	75	100	0	100
IL-10 levels (pg/mg protein)*	0.00 (0.00)	0.20 (0.50)	2.29 (0.68)	0.00 (0.00)	1.59 (1.30)	2.16 (0.72)	0.00 (0.00)	2.14 (0.74)
% of patients with IL-10 levels > 1.0 pg/mg protein	0	17	100	0	75	100	0	100

*Mean (SD).

patients and healthy controls. Large numbers of inflammatory cells in the connective tissues and connective tissue cells per se (ie, fibroblasts and endothelial cells) can lead to the release of IL-6, which is stimulated by bacterial products and by interaction with the host cells.[19,20,53] Bartold and Haynes[23] observed more intense IL-6 staining in histologic sections of inflamed human gingiva than in sections of healthy gingival tissue. Furthermore, Matsuki and associates[24] observed prominent IL-6 mRNA-expressing cells in the inflamed gingival tissue. These reports demonstrate that IL-6 levels are higher in diseased tissue than in healthy tissue, as observed in the present study.

Some controversy exists in the scientific literature concerning the sources of cytokines in saliva. Recent evidence has shown no correlation between IL-6 levels in serum and saliva.[54,55] Findings in a recent study support the contention that salivary IL-6 is produced locally by acinar and/or ductal cells in the salivary glands.[56] In addition, a difference in kinetics was also found for the IL-6 response in saliva and serum, indicating specific mechanisms for IL-6 production in saliva.[57] It is believed that salivary IL-6 reflects the response of the mucosal immune system.[55] A study by Seymour and colleagues[58] demonstrated that inflammatory cytokines in whole saliva might be derived from GCF. Increased levels of several cytokines, such as IL-1, IL-2, IL-6, IL-8, and TNF-α, have been observed in the GCF of patients with periodontal disease.[59] The penetration of bacteria and/or bacterial products into the tissues results in recruitment and activation of the monocyte/T lymphocyte axis. This leads in turn to the enhanced monocytic release of TNF-α, IL-1β, and IL-6, which have been associated with periodontal tissue destruction.[59] In conclusion, one may say that IL-6 is produced locally by tissue cells in response to an inflammatory stimulus and by salivary gland cells, reflecting the response of the mucosal immune system. Despite the source of the salivary IL-6 and IL-10, it is appropriate to assess immunologic patterns relevant to systemic or local disease conditions.[60–62]

It is normal that certain levels of IL-6 could also be detected in patients with clinically healthy peri-implant tissues. It is a well-established fact that small numbers of macrophages and mononuclear cells are usually present in clinically healthy gingival tissues.[63] These cells, as well as resident fibroblasts and endothelial cells, could synthesize and release IL-6.

In the present study, the level of IL-6 in the peri-implant disease group was positively correlated with PPD, BOP, and GI. These data suggest that a significant relationship exists between the amount of a proinflammatory cytokine (IL-6) and the destruction of peri-implant tissue. Thus, the presence of elevated levels of IL-6 in the saliva of patients with peri-implant disease, along with the significant correlation with clinical assessments of peri-implant tissue destruction, strongly suggests an important role for this mediator in the pathogenesis of peri-implant disease.

In the present study, IL-10 was detected only in saliva of patients with peri-implant disease; it did not appear at detectable levels in saliva of healthy controls. It is known that IL-10 inhibits bone resorption by suppressing cyclooxygenase-2-dependent prostaglandin E2 synthesis.[64,65] IL-10 also inhibits recruitment of osteoclast precursors and their differentiation to mature multinucleated osteoclasts.[66,67] IL-10 response could be due to a specific Th1/Th2 response.[68] In patients with periodontal disease, activation of an immune response by *Porphyromonas gingivalis* produces reactive T cells, which respond by secreting IL-10.[69,70] IL-10 is a cytokine-inhibiting factor that regulates the production of proinflammatory cytokines such as IL-1β, IL-6, IL-8, TNF-α, and INF-γ.[23,71–73] Therefore, the elevated levels of IL-10 in patients with peri-implant disease could perform a relevant role in the regulation of local immune response.[74] The degree to which IL-10 is detectable depends on the ability of the more potent integrated immune response to try to maintain inflammatory balance. For example, as IL-10 is able to suppress excessive oxidative burst,[75] its concrete levels in saliva reflect the tissue's counteraction of profound oxidative stress events, known as significant factors in peri-implant disease.[76]

Expression of IL-10 and some other cytokines has been demonstrated to be time-dependent. Schierano and coworkers[77] assessed cytokine expression at different time-points after implant placement. The results indicated that IL-10 expression increased at 4 months, decreased at 8 months, and became almost undetectable at 12 months, suggesting that this cytokine helps regulate immuno-inflammatory balance in peri-implant mucosa. In the present study, all the patients had had their endosseous implants in function for 61 to 72 months (mean, 67.2 ± 3.9 months).

CONCLUSIONS

- Data from the current study indicated that elevated levels of IL-6 and IL-10 were statistically associated with positive BOP, GI ≥1, and PPD > 3 mm.
- This investigation indicated that IL-6 and IL-10 levels are elevated in saliva of patients with peri-implant disease as compared to healthy patients.
- The data suggest that IL-6 levels > 1.5 pg/mg protein and IL-10 levels > 1.0 pg/mg protein may be used as the basis of a diagnostic test for peri-implant disease in patients with implant-supported overdentures.

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