Detection and Measurements of Soluble Intercellular Adhesion Molecules at Implants and Teeth: A Comparative Study

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ABSTRACT

Background: Adhesion molecules on endothelial cells and in the periodontal tissues control the immigration and retention of cells. The level of soluble intercellular adhesion molecules (sICAMs) has been used as a marker of the severity and/or extent of the inflammatory process in a wide range of pathologies, including periodontitis.

Purpose: This study was designed to detect and compare sICAM-1 at teeth and implants in relation to clinical periodontal and periimplant parameters.

Method: Regular recall patients with (1) implants and teeth, (2) implants, and (3) teeth were examined. Samples of sulcus fluid were collected from teeth and implants. The concentration of sICAM-1 was measured by enzyme-linked immunoabsorbent assay. Periodontal parameters were recorded after sampling.

Results: The range of measured sICAM-1 was large (from below 100 to 1,200 ng/mL). The concentration of sICAM-1 was not different for teeth and implants but was significantly elevated in sites with positive bleeding on probing (BoP), namely, 571 ng/mL at teeth and 529 ng/mL at implants compared with 150 ng/mL and 169 ng/mL, respectively, with negative BoP. The regression analysis showed that the concentration of sICAM-1 was highly associated with positive BoP but was not dependent on the fluid volume.

Conclusions: A similarity of the sulcus fluid at teeth and implants was observed with regard to the detection of sICAM-1.

KEY WORDS: implants, periodontitis, periimplantitis, sICAM, teeth

Periimplantitis is defined—in analogy to periodontitis—as an inflammatory reaction affecting the tissues of dental implants, resulting in bone loss. Soft tissue inflammation, bleeding on probing (BoP), suppuration, increased probing depth (PD), and microbiologic alterations in the flora mimic those signs associated with periodontal or periimplant disease.[1–3] The microflora around natural teeth and implants was found to be analogous.[1,4–7] Animal experiments have shown that the establishment of an experimental mucositis and periimplantitis follows a pattern very similar to that of natural teeth.[8,9] PDs, BoP, and attachment level (AL) are clinical parameters that may provide information regarding inflammatory processes with tissue loss around teeth. Measurements by means of these parameters are regularly applied for implants. However, they do not predict the progression of disease or identify sites at risk of deterioration.[10] It was suggested that the analysis of host factors in the sulcus fluid of teeth and implants, along with the anatomic events of periodontitis and periimplantitis, could be a useful tool for identifying disease progression.[11,12] Cellular and biochemical mediators in the sulcus fluid reflect the metabolic status of periodontal and periimplant tissues.[13,14] Adhesion molecules on endothelial cells and within the tissue itself control the immigration into and the retention of cells within

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the periodontal tissues.[15] Adhesion molecules, such as soluble intercellular adhesion molecule 1 (sICAM-1), are expressed in epithelia adjacent to implants in fashion similar to that around teeth.[16] Until now, the sICAM-1 level has been used as a marker of the severity and/or extent of the inflammatory process in a wide range of pathologies, such as autoimmune, malignant, and inflammatory diseases and periodontitis.[17,18]

The aim of the present clinical study was to detect and measure sICAM-1 of periimplant sulcus fluid (PISF) compared with gingival crevicular fluid (GCF) and to assess the concentration of sICAM-1 in relation to clinical periodontal parameters. The null hypothesis was that no significant differences will be found between measurements at teeth and implants.

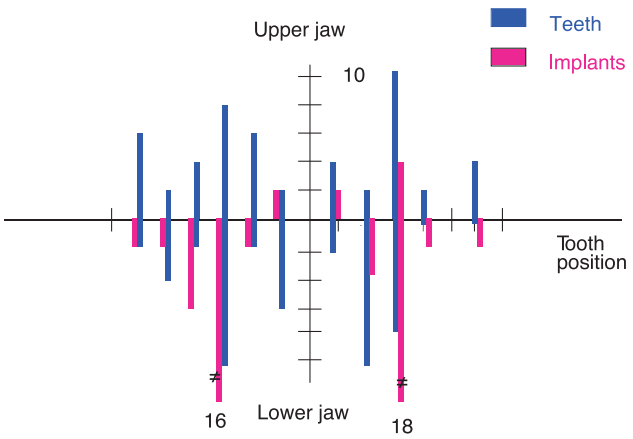

Figure 1 Distribution of implants and teeth in the mandible and maxilla.

METHOD

Patients

Regular recall patients, 29 females and 19 males, at the Department of Prosthodontics, School of Dental Medicine, Bern, Switzerland, with ITI dental implants (Straumann AG, Waldenburg, Switzerland) and/or with natural teeth were randomly selected and gave informed consent to participate in this study. The selection was performed by computer-randomized numbers based on the patients' chart numbers. The implants had successfully supported their prostheses for at least 24 months. The age of the patients at the time of the study ranged between 25 and 83 years, with a median age of 58.125 $\pm$ 11.2 years. Exclusion criteria were any systemic conditions that placed an individual in a high-risk category or would affect the inflammatory marker measurements (pregnancy, diabetes, hypertension, any inflammatory diseases, and use of antibiotics within the preceding 3 months or anti-inflammatory medication).

The patients were healthy and had confirmed by signature that they were nonsmokers or had stopped smoking before the implant therapy was started. They had performed good oral hygiene throughout the observation period at the department. They exhibited no or only mild signs of periodontitis, with occasional PDs of 5 to 6 mm. They had received neither professional hygiene procedures by the dental hygienist within the last 4 to 6 months before data collection for this study, nor any specific therapy owing to acute manifestation of periodontitis or periimplantitis. Based on the selection criteria, 48 patients were eligible for the study and were selected. The patients were allocated into three test groups with 16 patients each: (1) mixed group: one or more implants and teeth in the same jaw; (2) implant group: intraforaminal implants supporting an overdenture; and (3) teeth group: all natural teeth in both jaws. Figure 1 shows the distribution of implants and teeth in the maxilla and mandible.

Gingival Crevicular Fluid and Periimplant Sulcus Fluid

In the present study, known protocols for collecting crevicular fluid were adopted.[19] GCF and PISF were collected with sterile paper strips (Periopaper® strips, Pro Flow, Amityville, NY, USA). Samples were obtained at four sites in duplicate from all selected teeth and implants. Prior to the sampling, the area was gently dried with an air syringe and isolated by cotton rolls. If any obvious plaque was present on the visible surface of the implants and teeth, it was carefully removed with a curette. Strips contaminated with blood were discarded. After collection, the strips were immediately placed into a sterile Eppendorf tube containing 250 µL of ice-cold buffered saline consisting of 0.01 M Na H_2PO_4 and 150 mM NaCl, pH 7.4. The fluid was eluted from the paper strips by vortexing the samples for 30 seconds and was centrifuged at 800 *g* for 10 minutes. The supernatant was removed from the vials and was kept at $-80°C$ until analysis. The volume was expressed in microliters. Second, another Periopaper strip was inserted into the crevice and left in situ for 30 seconds and was then transferred to the chairside-located Periotron® 6000 measuring machine for volume

determination (Harco Electronics, Winnipeg, MB, Canada). Previously, it had been calibrated with known volumes of buffered solution.

sICAM-1 Assay

The amount of sICAM-1 in GCF samples was measured with an enzyme-linked immunoabsorbent assay (ELISA, human sICAM-1 Module set, high sensitivity kit, Benderer MedsysteM, Vienna, Austria). The sensitivity of ELISA is reported to be below 40 ng/mL. Concentrations were assayed in supernatant without dilution. The amount of crevicular sICAM-1 in each sample was determined from sICAM-1 standard calibration curves in nanogram/milliliter. The calibration curve was plotted by regression analysis, and the optical density of each sample was used to estimate the concentration of sICAM-1 in nanogram/milliliter. With regard to the sICAM-1 concentration from each sample, two values were obtained: the concentration was measured with the 250 mL buffer solution and the concentration was calculated according to the GCF and PISF volume as determined by the standard calibration curve.

Periodontal and Periimplant Parameters

The clinical parameters, including PD, AL, and dichotomous evaluation of BoP, were measured after the sampling procedure. They were recorded again at four sites of teeth and implants in each subject. PDs were measured from the gingival/periimplant mucosal margin to the bottom of the pocket with a calibrated probe. BoP was recorded with a plus sign if present and a minus sign if absent. AL is the sum of the PD and the positive or negative distance from the free margin of gingiva or mucosa to a defined landmark on teeth or implants.

Clinical measurements were performed at implants and teeth as follows:

1. Mixed group: one implant and the tooth in the corresponding position of the same jaw
2. Implant group: both intraforaminal implants were used for measurements
3. Teeth group: two teeth were randomly selected (computer-randomized numbers)

Shallow pockets and absence of BoP are used as parameters that indicate periodontal or periimplant stability. Increased PD and particularly BoP, or a combination of both, are considered to be indicators of an inflammatory process. Therefore, for further comparison, three groups of risk categories according to the periodontal parameters BoP and PD were established:

Risk l: PD $\leq$ 3 mm and negative BoP: heathy conditions, low risk

Risk II: PPD $\geq$ 5 mm and positive BoP: periodontal/periimplant process, high risk

Risk III: mixed findings; either PD $\leq$ 4 mm and positive BoP or PD $\geq$ 4 mm and negative BoP, not clearly attributed to high or low risk

Statistical Analyses

Two independent and blinded investigators were involved in the data collection: one did the sampling procedure with the paper strips and one measured the periimplant parameters. All data were processed in the computer, and statistics were performed using the *SAS* system program package (SAS Institute Inc., Cary, NC, USA). Power analysis revealed that at least 90 records should be available per group. This was obtained by measurements of 128 sites per group.

Means and standard deviations were compared between groups and subgroups by nonparametric testing (Mann-Whitney *U* test). Regression analysis was applied with the dependent variable sICAM-1.

RESULTS

Three hundred eighty-six sites from 48 implants and 48 teeth were available for measurements. The individual range of concentration of sICAM-1 was high. It was below 100 ng/mL in 48% and from 200 ng/mL up to $\geq$ 1,200 ng/mL in 52% of all sites. No values were identified between 100 and 200 ng/mL. Table 1 provides a comparison of the three test groups. Patients with only natural teeth (group 3) were significantly younger ($p < .05$), and the AL exhibited significantly lower values in the group with two intraforaminal implants. Of all PD measurements, 89.5% were below 4 mm. The percentage of sites with positive BoP was about 55% in all three groups.

Altogether, no major differences were found between implants and teeth, when comparing groups 2 and 3 or within group 1. The mean concentration of sICAM-1 specified for sites with positive BoP was 671.6 $\pm$ 549 at teeth and 529.7 $\pm$ 510 at implants compared with negative BoP with 150.8 $\pm$ 199 and 168.0 $\pm$ 189 at teeth and implants, respectively. This difference was

TABLE 1 Clinical Measurements in 3 Groups

	Group 1		Group 2		Group 3		Total	
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (yr)	59.8	8.4	61.87	7.1	52.7*	14.7	58.1	10.2
BoP (%)	55		53		58		55	
PD	2.61	1.6	2.42	1.3	2.24	1.0	2.42	1.3
AL	2.95	2.2	2.08*	1.7	2.85	2.2	2.63	2.06
sICAM-1								
Teeth (ng/mL)	374.1†	447			417.4	529	409.4	500
sICAM-1								
Implant (ng/mL)	380.14†	477	349.3	454			350.8	452
GCF (μL)	1.0	0.8	1.1	0.7	1.1	0.9	1.07	0.8

AL = attachment level; BoP = bleeding on probing; GCF = gingival crevicular fluid; PD = probing depth; sICAM-1 = soluble intercellular adhesion molecule 1.
Group 1: mixed group with an implant and corresponding tooth; group 2: 2 intraforaminal implants; group 3: natural teeth.
*$p < .05$ (Wilcoxon matched-pair signed rank).
†In mixed group.

statistically significant ($p < .05$). Accordingly, Figure 2 shows that 70% of all measured sites with negative BoP exhibited a concentration of sICAM-1 below 100 ng/mL, whereas this was observed in only 27% of all measured sites with positive BoP. Table 2 represents the findings of the three risk groups. All 15 sites belonging to risk group 3—about 4%—were found in 12 different patients. A match of implant and tooth sites was observed. Concentration of sICAM-1 at sites with increased PD and positive BoP was significantly higher, as shown by the comparison of risk groups 1 and 3. In the mixed group, the concentration of sICAM-1 was statistically significantly higher than in risk group 1. The majority of these sites had positive BoP, whereas PD was mostly ≤ 4 mm in this mixed group.

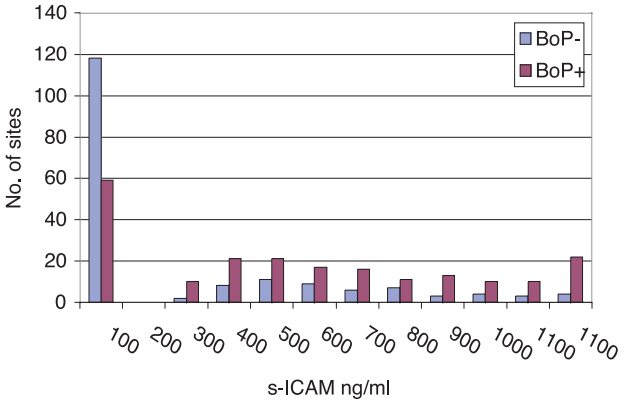

Figure 2 Concentration of soluble intercellular adhesion molecule (sICAM) in intervals of 100 ng/mL and corresponding number of sites with positive or negative bleeding on probing (BoP).

The results of the regression analysis are given in Table 3. A strong association was found only between the concentration of sICAM-1 and positive BoP. The regression analysis was consistent with the results obtained from the comparisons of the risk groups.

DISCUSSION

The present study confirms that sICAM-1 can be detected and measured equally in the sulcus fluid of teeth and implants, with a wide individual range. Further, the concentration is elevated in the presence of positive BoP. The age range between the group 3 (only teeth) and group 2 (only implants) was slightly different. Therefore, for further comparison of teeth and implants, the mixed group with implants and teeth in the same jaw was also introduced in the study. Some researchers emphasized the similarity of periodontal and periimplant tissues regarding the inflammatory process at implants and teeth.[15,16,20] Other researchers had some doubts as to whether the periodontal and periimplant tissues should be considered a comparable entity regarding structure and function. The first one is a highly specialized developmental tissue, whereas the second one is a scar tissue of wound healing.[21] In the present study, the amount of volume of GCF and PISF was measured with the Periotron

TABLE 2 Clinical Data in 3 Risk Groups

	Risk Group 1			Risk Group 2			Risk Group 3	
Variable	$n^{\dagger}$	Mean	SD	$n^{\dagger}$	Mean	SD	$n^{\dagger}$	Mean	SD
Age (yr)	153	56.5	13.2	216	59.21	9.8	15	58.2	5.8
BoP (%)	153	0		216	91		15	100	
PD	153	1.84	0.9	216	2.58	1.2	15	6.0	1.2**
AL	153	2.11	1.6	216	2.78	2.0	15	5.66	2.4**
sICAM-1 (ng/mL)	153	142.5	226.7*	216	524.17	508.3	15	729.4	675.1
GCF (μL)	153	0.78	0.4	216	1.28	0.9	15	1.1	0.9

AL = attachment level; BoP = bleeding on probing; GCF = gingival crevicular fluid; PD = probing depth; sICAM-1 = soluble intercellular adhesion molecule 1.
Risk group 1: PD ≤ 3 mm and negative BoP; risk group 2: sites with mixed findings; risk group 3: PD ≥ 5 mm and positive BoP.
*$p < .01$; **$p < .001$ (Wilcoxon matched-pair signed rank).
†Number of measured sites per group.

6000, which was calibrated with known volumes of buffered solution. It is debatable whether or not such volume measurements are reliable and important. In healthy sites, collecting crevicular fluid is difficult. In fact, for both implants and teeth, no association was found between the measured volume of sulcus fluid and the concentration of sICAM-1. It appears that the concentration of sICAM-1 as measured by means of the buffer solution is more important than its concentration relative to the volume of GCF or PISF.

It has been demonstrated that the soluble forms of ICAM-1 can be detected and assayed in GCF in both healthy or pathologic periodontal patients and that higher levels of this molecule are noted in patients with plaque or inflammation.[18] One study used only samples from tooth sites with at least 5 mm PD.[20] Because the authors did not provide information about the coincidence of increased BD, positive or negative BoP, and the respective concentration of sICAM-1, the association between these three parameters is not clear from their findings. It was also demonstrated that the serum concentration of sICAM-1 was significantly elevated in smokers compared with nonsmokers,[19] whereas the crevicular fluid did not exhibit an analogue increase of concentration of sICAM-1.[19,22] The influence of smoking could not be investigated in the present study because it was an exclusion criterion; neither were serum measurements available. This is a limitation in the significance of the present study. Nevertheless, the mean values of sICAM-1 concentration as measured in the present study, although slightly higher, were comparable to the results mentioned above. Our findings also show that BoP might be a better criterion for an inflammatory process—as represented by the marker sICAM-1—than increased PD.

TABLE 3 Regression Analysis with Dependent Variable Concentration of Soluble Intercellular Adhesion Molecule 1

Independent Variable	Mean Square	*p* Value
Patient age	23,653.10	.62
Gender	21,0637.2	.59
Teeth/implants	5,394.70	.81
Location in jaw	291,637.50	.08
Probing depth	364,615.90	.05
Attachment level	277,859	.09
Bleeding on probing (+)	487,519.00	.02*
GCF/PISF (μL)	143,995.90	.22

GCF = gingival crevicular fluid; PISF = periimplant sulcus fluid.
*$p < .05$.

Various studies analyzed whether measurements of BoP and PD are useful as predictors of disease activity.[23,24] However, none of these studies revealed the specificity of periodontal indices for periimplant soft tissue based on a long-term controlled follow-up. A recent review exhibits controversy on this topic.[25] It was suggested that the absence of BoP be used as a criterion for stability rather than a predictor of disease activity.[26–28] From a clinical point of view, absence of BoP around implants would then indicate healthy periimplant tissues. The degree of bleeding (single small spots, moderate or heavy profuse bleeding) is not discriminated by this index but could reflect

various degrees of inflammatory status. The combination of increased PD ($\geq$ 5 mm) and positive BoP may be a better indicator of periodontitis and peri-implantitis.[27–29] If sites with PD > 4 mm combined with positive BoP were compared with sites exhibiting PD $\leq$ 3 mm in combination with negative BoP, a significantly higher concentration of sICAM-1 at teeth and implants was found. Sites allocated into the mixed risk group mostly exhibited positive BoP combined with shallow pockets. Here again, a significantly higher concentration of sICAM-1 was found compared with sites with negative BoP.

If positive BoP and a high concentration of sICAM-1 are both indicators of inflammation, then the coincidence observed should not be surprising. Our findings indicate that the number of sites with positive BoP was elevated, with an average of 55% at teeth and implants. This might contrast to the fact that all patients had regularly followed a maintenance care program, and it was considered that they did not exhibit teeth and implants at high risk. In the present study, a force calibrated probe was not used. High probing forces (> 25 g) can evoke BoP, causing tissue damage. If positive BoP was only the consequence of high probing forces, this would then not explain the association with sICAM-1. One could speculate that high probing forces would evoke bleeding only at sites with some processes of inflammation, which are not yet detectable by clinical parameters. Still, the capacity of these parameters to discriminate between healthy and active sites seems to be limited. Similar observations were made by investigators of various markers in the sulcus fluid compared with periodontal parameters.[30]

From the present investigation, the null hypothesis has to be accepted. We have shown a high similarity between teeth and implant sites with regard to the detection and measurements of sICAM-1 and an association between the marker sICAM-1 and positive BoP. The significance of this observation should be investigated by long-term observations.

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