

CrossLaps and β -glucuronidase in Peri-implant and Gingival Crevicular Fluid

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Collagen degradation products of the carboxyterminal region possibly reflect bone and attachment loss. In the present study, the Serum CrossLaps One-Step enzyme-linked immunosorbent assay was used to determine a specific part of the carboxyterminal region of type I collagen, the CrossLaps. Samples of peri-implant and gingival crevicular fluid of 111 implants and 53 teeth from 47 partially or completely edentulous patients were examined in reference to levels of CrossLaps and β -glucuronidase (β G), an established marker of periodontal disease. Clinical probing pocket depth (PPD), bleeding on probing (BOP), plaque accumulation, mobility, radiographic bone loss, and the occurrence of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia* were assessed. The mean values were: for PPD at implants 3.76 ± 1.41 mm, at teeth 3.44 ± 0.88 mm; for β G at implants 0.364 ± 0.392 pU/min, at teeth 0.314 ± 0.209 pU/min; for CrossLaps at implants 0.069 ± 0.059 pmol/min, at teeth 0.082 ± 0.053 pmol/min. Bleeding on probing was significantly higher on implants than on teeth (McNemar test, $P = .004$). No significant difference of β G levels was found between teeth and implants (Wilcoxon test). A negative correlation was found between β G levels and CrossLaps levels at teeth (Pearson-rank correlation, $P = .002$). On implants, no significant correlation of these 2 parameters was seen, but significant correlations were found between sulcus fluid flow rate and PPD ($P = .012$), β G levels and bone loss ($P < 0.0005$), and CrossLaps levels and PPD ($P = .011$). CrossLaps can be detected in both gingival and peri-implant crevicular fluid. While rising levels of β G may indicate acute peri-implantitis, CrossLaps may not, but could play a role as a marker of ongoing attachment loss. (INT J ORAL MAXILLOFAC IMPLANTS 2001;16:252-258)

Key words: collagen, dental implants, enzyme-linked immunosorbent assay, gingival crevicular fluid, peri-implantitis, Serum CrossLaps

Gingival crevicular fluid (GCF) has often been used for the assessment of periodontal disease. Analysis of components of gingival crevicular fluid, such as β -glucuronidase (β G), have been suggested as markers for marginal periodontitis. Bang and others¹ found a correlation between the severity of

periodontal lesions and the activity of β G. Lamster² proposed that β G as a marker for release of lysosomal granules by neutrophils reflects the damage of periodontal tissues by enzymes. Lamster and others³ showed that increased levels of β G were strongly associated with progressive clinical attachment loss. Later, β G levels were measured in peri-implant crevicular fluid (PICF) by Kleber and colleagues⁴ with a similar procedure.

Degradation products of collagen have also been found in GCF. Carboxyterminal telopeptides are nonhelical regions of the collagen molecule that are cross-linked to helical regions of other collagen molecules. Since carboxyterminal telopeptides cannot be degraded by proteases, their presence can be detected in bodily fluids.⁵ Talonpoika and Hämäläinen⁶ measured the carboxyterminal telopeptide of type I collagen (ICTP) in gingival crevicular fluid. These authors considered the carboxyterminal telopeptide to be a marker of the degradation rate of collagen type I in periodontal tissue. Oringer and

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coworkers⁷ demonstrated ICTP in the peri-implant sulcus. In addition, ICTP can be detected by radioimmunoassay.

Previously, an 8-amino-acid fragment of the carboxyterminal region of collagen type I, which has been shown to be a sensitive index of the rate of bone resorption, was detected in urine using a non-radioactive test, the CrossLaps One-Step enzyme-linked immunosorbent assay (ELISA) (Osteometer BioTech, Herlev, Denmark).^{8,9} An improved version to measure fragment levels in serum, the Serum CrossLaps One-Step ELISA,¹⁰ has been applied for the first time in the present study to detect CrossLaps levels in crevicular fluid.

The aims of the present study were:

1. To ascertain whether the Serum CrossLaps ELISA can be used for analysis of gingival and peri-implant crevicular fluid,
2. To find correlations between CrossLaps and other clinical parameters at implants and teeth, and
3. To search for differences between implants and teeth regarding CrossLaps levels.

To relate the CrossLaps values to an established marker of progressive periodontitis, β G activity was also determined in the crevicular fluid.

MATERIALS AND METHODS

Subjects

One hundred eleven endosseous dental implants and 53 teeth from 47 partially and completely edentulous patients were examined. The patient population consisted of 19 men and 28 women who took part in the study during their regular checkup. The mean age was 51.5 years (range, 21 to 77 years). Exclusion criteria were acute systemic diseases, acute pathologic symptoms in the oral cavity, current medication with antibiotics, and pregnancy.

Several different implant systems were included: 66 Frialit-2 implants (Friadent, Mannheim, Germany); 19 IMZ implants (Interpore International, Irvine, CA); 11 Astra implants (Astra Tech, Mölndal, Sweden); 6 Brånemark System implants (Nobel Biocare, Göteborg, Sweden); 3 ZL implants (ZL Microdent, Breckerfeld, Germany); 3 ITI Bonefit implants (Straumann, Waldenburg, Switzerland); 2 blade implants (Oraltronic, Bremen, Germany); and 1 3i implant (Implant Innovations Inc, Karlsruhe, Germany). The time since implant placement ranged from 12 to 72 months (mean 41.7 months). All implants had been loaded after a healing period of 6

months in the maxilla and 4 months in the mandible. Various types of superstructures were used. Partially edentulous patients were treated with implant-supported crowns and partial prostheses, while edentulous patients received bar-retained overdentures.

Sampling of Crevicular Fluid

Peri-implant fluid and GCF samples were collected using Periopaper strips (ProFlow, Amityville, NY). In edentulous patients, only the GCF of the implants could be sampled (54 implants). In partially edentulous patients, samples of GCF were obtained from the implants and the contralateral corresponding teeth (57 implants and 53 teeth). On each implant and each tooth, samples were obtained from the mesial and the distal. Sampling was performed according to the method of Brill and Krasse.¹¹ The paper strip was put into the crevice until resistance was felt and left there for 1 minute.

The sulcus fluid flow rate was measured by Periotron 6000 (Harco Electronics, Winnipeg, Alberta, Canada). The Periotron had been calibrated with known volumes of human serum, and before each sampling, calibration to zero was done with a dry strip. The Periopaper strips were immediately placed into a solution of bovine serum albumin (1%) in 0.15 mmol/L sodium chloride. They were shock-frozen in liquid nitrogen, kept at -20°C , and thawed immediately before the detection of CrossLaps and β G. The elution of CrossLaps and β G in GCF took place by means of a Vortex-Mixer (Unimag ZX, Uni Equip, Martinsried, Germany). The tubes were shaken 3 times for 30 seconds at intervals of 10 minutes apart and subsequently centrifugated at $20,000\times g$.

CrossLaps and β -glucuronidase Determination

Determination of the CrossLaps was performed with the Serum CrossLaps ELISA kit (Osteometer BioTech). Biotinylated antibody and peroxidase-conjugated antibodies form a complex with the CrossLaps antigen that binds to streptavidin. A color reaction occurs in the presence of a chromogenic substrate. Absorbance is measured at 450 nm. In the present study, rather than serum, 100 μL gingival fluid extract was mixed with 100 μL antibody solution. The same procedure was repeated with 100 μL of the same GCF sample as double detection. The recovery rate for determination of CrossLaps was $80.6\% \pm 3.7\%$, which resulted from the use of varying standard concentrations of the CrossLaps with the described method. The concentration corresponding to 3 standard deviations above the mean of 10 determinations of the blank standard, the sensitivity, was evaluated as 0.006 pmol. The variation coefficient was $\pm 5\%$.

The activity of β G was estimated by the method of Hall and colleagues.¹² 4-Nitrophenyl- β -glucuronid is hydrolyzed by β G to 4-nitrophenol, which is measured spectrophotometrically at 400 nm. As a modification of the method of Hall and colleagues, the incubation was performed at 56°C for 2 hours. For the determination of β G, the recovery rate was $86.4 \pm 4.1\%$, the sensitivity was $0.015 \mu\text{U}$, and the variation coefficient was 5%.

Clinical Monitoring

Prior to follow-up, measuring, and sampling procedures, all patients received a description of this investigation approved by the Ethics Committee of the Medical Faculty of Charité. Clinical assessments included probing pocket depth (PPD) measured by a periodontal probe with controlled force (Aesculap, Tuttlingen, Germany); bleeding on probing (BOP); plaque accumulation according to Silness and L oe¹³; and width of attached gingiva. DNA probes (Wybert, Lorrach, Germany) were used for semi-quantitative determination of the periodontal pathogens *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia*. The mobility of implants and teeth was determined by the Periotest device (Siemens, Bensheim, Germany). Radiographic bone loss around implants was estimated by comparison of sequential radiographs: panoramic radiographs were made, and measurements of bone loss were made using the distance from a reference point (implant shoulder) to the margin of the bone. Values were compared with the radiographs taken immediately after implant placement. Radiographic distortions were compensated by considering the implants as reference subjects with known length and diameter.

Statistical Analysis

For each implant and tooth, data from mesial and distal sites were available. For statistical calculation, the mean values were used.

Multiple observations from the same subject were seen as independent, because this study regarded mainly local factors of development of periodontitis and peri-implantitis. All implants were compared to all teeth. The influence of interindividual differences was taken into account in a second part of the study, and the corresponding data will be published in a subsequent paper.

Since data were non-normally distributed, non-parametric tests were used. Statistical analysis of differences in measurements between implants and teeth was performed with the Wilcoxon test. Correlations between clinical and biochemical parameters at implants or at teeth were determined by calculation of

Pearson correlation coefficient. Two groups were distinguished: implants and teeth with PPD up to 3 mm, and those with PPD over 3 mm. Differences concerning the clinical and biochemical parameters of both groups were assessed by the Mann-Whitney test and the Chi-square test. Clusters of all parameters were assembled by factor analysis. The Varimax rotation method with Kaiser normalization was used. Included parameters were plaque accumulation, BOP, PPD, sulcular fluid flow rate (SFFR), β G, and CrossLaps for implants and teeth; in addition, bone loss, levels of *P gingivalis* and *P intermedia*, and Periotest values were included for implants. Factor analysis for implants and teeth took place separately. SPSS 8.0 software (SPSS, Chicago, IL) was used for all statistical analysis.

RESULTS

Mean values and standard deviations, as well as minimum and maximum levels of all metric parameters, are shown in Table 1.

When the clinical parameters between implants and teeth were compared, significantly greater PPD ($P = .013$) and SFFR ($P < .0005$) were found for implants. However, no significant differences between the concentration of CrossLaps and the activity of β G were found between implants and teeth (Fig 1). Bleeding on probing occurred significantly more frequently on implants than on teeth (McNemar test, $P = .004$).

Correlation coefficients between the different parameters determined at implants are shown in Table 2. For implants, there was a significant correlation found between SFFR and PPD, as well as between the concentration of CrossLaps and PPD. The activity of β G was significantly correlated with bone loss measured radiographically.

For teeth, there was also a significant correlation between SFFR and PPD (Table 3). β -glucuronidase was shown to correlate with both SFFR and PPD. A significant negative correlation was found between CrossLaps and β G. Periotest values showed a significant correlation to SFFR ($P = .008$), as well as to PPD ($P = .014$).

For implants with PPD greater than 3 mm, SFFR and concentration of CrossLaps were significantly higher in comparison to implants with probing pocket depths of 3 mm or less (Fig 2). Teeth with probing pocket depths greater than 3 mm revealed an increased level of β G (median level PPD < 3 mm: 0.235, PPD > 3 mm: 0.344; Mann-Whitney U test, $P = .014$).

Factor analysis was performed for investigation of possible connection between clinical and biochemical

Table 1 Values of Parameters Studied

Parameter	n	Mean	SD	Minimum	Maximum
Sulcus fluid flow rate (μL)					
Implants	111	1.178	0.541	0.160	2.080
Teeth	53	1.093	0.471	0.380	2.050
Probing pocket depth (mm)					
Implants	110	3.760	1.410	1.000	7.500
Teeth	53	3.440	0.880	2.000	6.000
β -glucuronidase ($\mu\text{U}/\text{min}$)					
Implants	110	0.364	0.392	0.005	1.995
Teeth	53	0.314	0.209	0.032	1.180
CrossLaps (pmol/min)					
Implants	110	0.069	0.059	0.012	0.397
Teeth	53	0.082	0.053	0.003	0.271

parameters. For implants, 3 clusters could be identified. One of these clusters consisted of 4 parameters: plaque accumulation, BOP, bone loss, and concentration of βG . The second cluster was formed by the levels of *P. gingivalis* and *P. intermedia* in subgingival plaque and SFFR. The third cluster included PPD, concentration of CrossLaps, and Periotest values (Fig 3). Three clusters were also found for teeth. The first was formed by plaque accumulation, BOP, and Periotest values. The second cluster comprised SFFR, PPD, and concentration of βG . CrossLaps levels formed the third cluster.

The appearance of inflammation around implants was not influenced by the width of attached gingiva.

DISCUSSION

Analysis of GCF components has been used previously for the purpose of finding a marker of periodontal disease that is more reliable and convincing than customary clinical parameters. Since there are scientifically verified similarities between microbiologic findings and the pathogenesis of periodontitis and peri-implantitis,¹⁴ the same clinical parameters (BOP, PPD, suppuration, and radiographic bone loss) are used for diagnosis of peri-implantitis. However, morphologic differences should be considered. The probing pocket depth around implants is generally greater than that around teeth because of the lack of periodontal connective tissue on implants. This finding of Ericsson and Lindhe¹⁵ was confirmed by the present study. A significantly higher rate of BOP around implants than around teeth was found. For implants, BOP may occur not only in inflamed sites but also in healthy sites.¹⁵

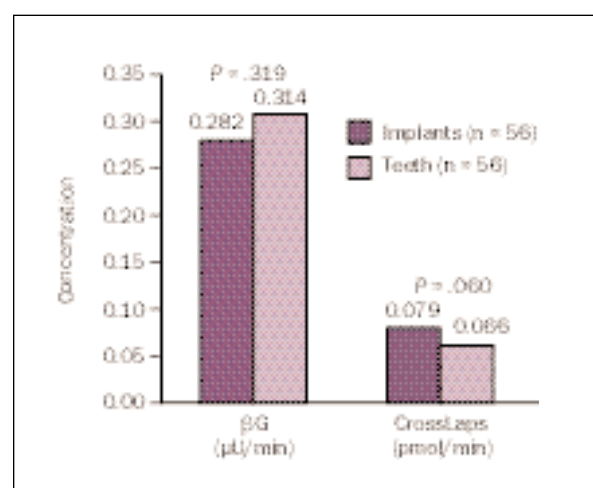


Fig 1 Comparison between implants and teeth concerning the concentrations of βG and CrossLaps (Wilcoxon test).

Measurements of the amount of PICF have also been carried out as a diagnostic method.¹⁶ In a study by Schatz and coworkers,¹⁷ SFFR and enzyme activities in implants and teeth were investigated and compared. The findings showed similar results of implants and teeth regarding SFFR, alkaline and acid phosphatase, βG , and aryl sulfatase. Enzyme activity was enhanced with increased SFFR.

In the present study, βG was analyzed, as it has been proven to be an indicator for the acute phase of periodontitis, correlating with customary clinical parameters such as BOP, bone loss, and SFFR.^{1,3} Lamster and associates¹⁸ showed that βG is a reliable indicator for prediction of attachment loss in chronic

Table 2 Correlations Between Parameters at Implants (Pearson Correlation Coefficient)

Parameter	Probing pocket depth	Bone loss	β -glucuronidase	CrossLaps	Periostest values
Sulcus fluid flow rate	$r = .238$ $P = .012^*$	$r = -.028$ $P = .779$	$r = .007$ $P = .944$	$r = .171$ $P = .074$	$r = .236$ $P = .014^*$
Probing pocket depth		$r = .144$ $P = .153$	$r = .099$ $P = .307$	$r = .242$ $P = .011^*$	$r = .258$ $P = .008^*$
Bone loss			$r = .377$ $P = .000^*$	$r = .118$ $P = .240$	$r = .116$ $P = .232$
β -glucuronidase				$r = .075$ $P = .439$	$r = -.064$ $P = .515$
CrossLaps					$r = .081$ $P = .411$

*Significant correlation.

Table 3 Correlations Between Parameters at Teeth (Pearson Correlation Coefficient)

Parameter	Probing pocket depth	β -glucuronidase	CrossLaps	Periostest values
Sulcus fluid flow rate	$r = .314$ $P = .019^*$	$r = .373$ $P = .006^*$	$r = -.019$ $P = .890$	$r = .121$ $P = .388$
Probing pocket depth		$r = .510$ $P = .000^*$	$r = -.213$ $P = .126$	$r = .381$ $P = .005^*$
β -glucuronidase			$r = -.368$ $P = .007^*$	$r = .231$ $P = .103$
CrossLaps				$r = -.060$ $P = .675$

*Significant correlation.

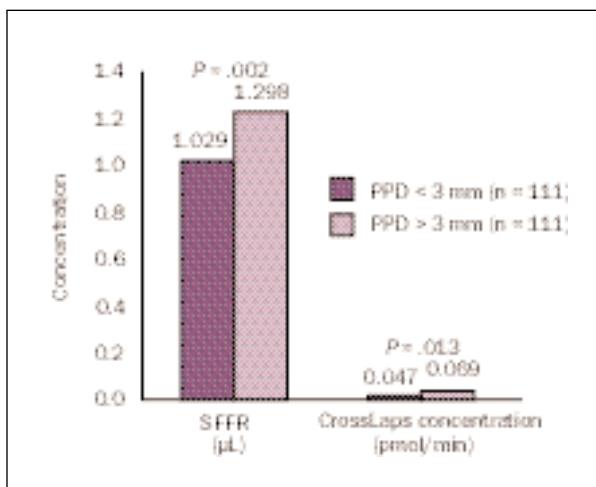


Fig 2 Comparison of implants with probing pocket depth (PPD) smaller or greater than 3 mm. SFFR = sulcular fluid flow rate.

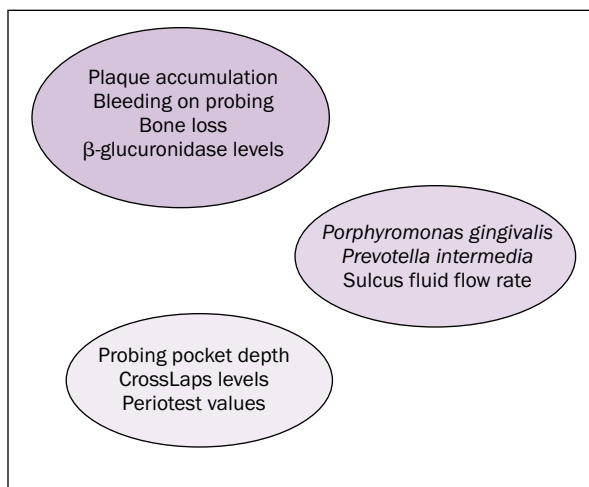


Fig 3 Graphic representation of the clusters of parameters at implants found with factor analysis.

periodontitis. The method of detection of β G applied in this study has been used successfully in previous studies.^{4,18–20} Raising the incubation temperature to 56°C can increase the sensitivity of the test.¹⁹

The results of the present study show a clear correlation of the level of β G and bone loss around implants, as well as between β G and SFFR and PPD around teeth. This underlines the importance of this enzyme in the diagnosis of both periodontitis and peri-implantitis.

In addition to the enzymatic component of GCF, collagen degradation products have been detected. One of the most specific markers of these degradation products is the ICTP.²¹ Carboxyterminal telopeptides have been found in GCF and in PICF^{6,7,22,23} using a radioimmunoassay. In the study of Talonpoika and Hämäläinen,⁶ total amounts of carboxyterminal telopeptide were positively correlated with clinical parameters. In the present study, the nonradioactive Serum CrossLaps One-Step ELISA was used. It determines a specific part of the carboxyterminal region of collagen type I, the CrossLaps consisting of an 8-amino-acid fragment. The ELISA used in this study was applied to investigate GCF for the first time. Various other studies have used the urinary and the Serum CrossLaps ELISA test for monitoring bone resorption and anti-resorptive therapies.^{8,9,10,24}

The results of the present study show that the Serum CrossLaps One-Step ELISA can be used for detecting specific collagen degradation products also found in gingival and peri-implant crevicular fluid. A slight tendency to higher levels of CrossLaps at implants, which could not be statistically proven, may be the result of the more progressive spreading of inflammation around implants, which is accompanied by a more distinct bone loss.²⁵

The findings of the study of Talonpoika and Hämäläinen⁶ could not be confirmed with these results. Only on implants did CrossLaps levels correlate with PPD. On teeth, a negative correlation was found between CrossLaps and β G levels.

CONCLUSION

It was concluded that β G may be a useful marker for peri-implantitis, as has been previously shown by other authors for periodontitis. However, the CrossLaps level did not correlate with customary clinical parameters. Further longitudinal investigations should examine whether CrossLaps levels in crevicular fluid may be used as a marker for ongoing attachment loss. Additionally, levels of CrossLaps should be evaluated at different stages of inflammatory periodontal disease.

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