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A Comparison Of Il-1 β , E2 (Pge2), Pai-2 and Tnf- A Levels in Gingival Crevicular Fluid (Gcf) and Peri-Implant Crevicular Fluid (Picf) from Groups of Patients with Healthy Teeth, Healthy Implants, Periodontitis and Peri-Implantitis

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Abstract

Objectives: The aim of this study was to analyse simultaneously four markers in gingival crevicular fluid (GCF) and peri-implant crevicular fluid (PICF) from 41 patients in four different groups over a period of six months in order to establish profiles indicating quantitative ratio and potential correlations.

Material and Methods: We compared levels of interleukin-1 beta (IL-1ß), prostaglandin E2 (PGE2), specific plasminogen activator inhibitor 2 (PAI-2) and tumor necrosis factor-alpha (TNF-α) in GCF and PICF from four groups of patients: healthy teeth (n=10), healthy implants (n=10), periodontitis (n=11) and peri-implantitis (n=10). Clinical parameters including bacterial flora were quantified by PCR-analysis. The concentration in GCF/PICF of IL-1β, PAI-2, E2 (PGE2) was determined by ELISA, TNF-α by Western blot analysis.

Results: Healthy teeth showed significantly higher levels of IL-1β than healthy implants. The mean level of E2 (PGE2) for healthy teeth was twice as high as for healthy implants. Periodontitis and peri-implantitis sites showed highly increased secretion of IL-1ß and E2 (PGE2) resulting in significantly higher levels of IL-1ß in periodontits than in peri-implantitis. Highest TNF-α levels showed in the peri-implantitis group, lowest in healthy teeth. Lowest concentrations of PAI-2 appeared in peri-implantitis, highest in healthy implants.

Conclusions: Within the limits of the study it could be hypothesized that the significantly higher levels of IL-1ß and E2 (PGE2) for healthy teeth than for healthy implants could in part be due to the lack of periodontal ligament cells around implants. Another possible explanation could be the anti-inflammatory property of titanium surfaces of implants.

Keywords: Gingival crevicular fluid (GCF); Peri-implant crevicular fluid (PICF); Periodontitis; Peri-implantitis

Introduction

The etiology of periodontal and peri- implant surrounding tissue structure and their host dependent immunological reactions are still not entirely clear. The levels of GCF / PICF inflammatory mediators are no doubt driven by a variety of genetic determinants. Microorganisms are important for initiating and sustaining periodontal disease and a prolonged inflammatory process is crucial for the destruction of the periodontal tissue [1,2]. For peri-implantitis a similar patho-mechanism is also possible but the lack of periodontal ligament (PDL) might cause alteration in inflammation and immunological reaction to tissue surrounding implants.

In order to identify markers defining periodontal status, analyses of gingival crevicular fluid (GCF) are essential. GCF is a serum transudate or "inflammatory" exudate found in gingival crevices or periodontal pockets around teeth [3]. It contains a variety of materials, including leukocytes, antibodies, complement proteins, enzymes, cytokines [4,5] as well as activators and inhibitors of the fibrinolytic system [6,7]. These maintain the homeostasis of periodontal tissue and are assumed to be part of the protective response against dental plaque bacteria.

Inflamed gingival and periodontal tissues contain and release numerous types of inflammatory mediators which are able to stimulate tissue degradation and bone resorption. Several molecules in GCF are present in inflammatory processes and have been found to stimulate bone resorption including cytokines like interleukin, (IL-1, IL-6, IL-11, IL-17), tumor necrosis factor (TNF-α,-β), macrophage colony stimulating factor (M-CSF), thrombin and prostaglandins E2 (PGE2).

However, IL-1a and IL-1ß may be the principal inflammaton-induced cytokines stimulating bone resorption in periodontitis [8-10]. In peri-implant crevicular fluid (PICF), higher levels of IL-1ß have been associated with peri-implantitis [11-15]. Furthermore, the fibrinolytic system, the plasminogen activator t-PA and its antagonist plasminogen activator inhibitor PAI-2 play a significant role in the balance of periodontal tissue destruction and remodelling [6,7] as a

possible protective component. PAI-2 supposedly exists intracellularly in a relaxed form in gingival tissue [16]. Plasminogen activator t-PA and inhibitors PAI-2 were found at high concentrations in gingival crevicular fluid and especially in areas of gingival inflammation.
For our study we selected four mediators found in GCF/PICF to be analysed and quantitatively compared within four groups of patients:

IL-1ß may be one the most important cytokines in the inflammatory process, a multifunctional mediator in acute and chronic periodontal disease. It is supposed to be a strong pro-inflammatory protein because it mediates the production of prostaglandin E2 (PGE-2) leukotrienes and platelet-activating factor in several cell types. These two stand in line of a cascade mechanism which promote osteoclast formation and bone resorption. The tumor necrosis factor-alpha (TNF-α) was also included in our study as well as the specific plasminogen activator inhibitor 2 (PAI-2) which could represent a possible protection of extracellular matrix degradation.

Method and Materials

Patient selection

Study subjects were randomly selected from patients referred to the Department of Prosthodontics at the Dental School, University of Tuebingen. All patients participating in the study gave informed written consent. Individuals who had a history of antibiotic treatment within the last 6 months or were taking other medications such as cyclosporin, phenytoin or Ca-channel blockers were excluded from the study; pregnant ladies, patients in active orthodontic treatment, individuals with a history of drug abuse or suffering from systemic diseases (e.g. diabetes mellitus, immunosuppressive illnesses, hematological disorders) were also excluded.

Patients showing sites of suppuration or patients with clinical or radiographic endodontic pathology, untreated caries, defect fillings and inadequate dental restorations were also excluded. For the purpose of this study only one tooth or implant (site) per patient was randomly selected for sulcus fluid analysis.

Periodontal disease was defined as probing depths ≥ 3 mm, attachment loss, clinical inflammation, bleeding on probing or radiographic evidence of bone loss.

Ten patients (7 females and 3 males) with healthy teeth, mean age 31 years (23-47) with no history of periodontal disease served as one control group, probing depths ≤ 3 mm.

Ten patients (7 females and 3 males) with healthy osseo-integrated titanium implants served as the second control group. Their mean age was 38 years (19-67) with healthy gingival tissue appearance, a mean time since loading the implants of 87 months (36-192) and probing depths ≥ 3 mm.

Ten patients (2 females and 8 males) with chronic periodontitis, mean age 49 years (26-73), with clinical signs of inflammation, bleeding on probing, bone loss with radiographic evidence, probing depths ≥ 5 mm were included in the study.

The last group consisted of eleven patients (6 females and 5 males) with chronic peri-implantitis, mean age 52 years (34-76), mean time since loading the implants = 36 months (12 - 73), defined as an inflammation process around implants with loss of bone, probing depths ≥ 5 mm [17].

Clinical measures

All registrations and samplings of GCF / PICF were performed during the same visit. GCF/ PICF was collected first, plaque probes second and then clinical parameters, probing depths (PD) were recorded. PD was defined as the distance in mm from the coronal-most margin of the free gingival to the most apical penetration of the Michigan-O probe. All measurements for the control groups were performed twice within 6 months to ensure reliable results. Plaque analysis was carried out using a commercially available polymerase chain reaction (PCR)-Kit, Micro Dent® (Hain Diagnostika, Nehren, Germany) in accordance with the manufacturer´s guidelines.

The gingival crevicular fluids GCF and peri-implant crevicular fluids PICF were collected at mesiobuccal and distobuccal sites of teeth / implants. Prior to sampling the respective region was dried by isolation with cotton rolls and a gentle air stream of 10 s duration with a 90 degree angle to the tooth axis [18]. Supragingival plaque was not removed.

A periopaper (Harco, New York, USA) was inserted into the gingival or peri-implant crevice until slight resistance was felt and then held in place for 60 s. Strips were measured for fluid volume by a serum equilibrated Periotron 6000 (Harco, New York, USA). The two strips of each tooth / implant were pooled and regarded as a whole sulcus sample. They were transferred to microcentrifuge tubes containing 100 µl PBS balanced isotonic solution (Invitrogen GmbH, Karlsruhe, Germany) then centrifuged and eluted in 25 µl aliquots and stored at -70°C until used in assays. Indometacin was added to aliquots for E2 (PGE2) analyses to avoid degradation. Samples contaminated with blood were rejected.

Plaque Sampling

Plaque sampling was performed using endopapers (Roeko, Size 40, Langenau, Germany), which were inserted at mesiobuccal and distobuccal sites most apical in the sulcus, held in place for 15 s and stored in separate microcentrifuge tubes at -70°C for subsequent polymerase chain reaction (PCR) analysis.

Analysis of GCF / PICF

Samples were analysed simultaneously using the same assay. Diluted samples were analysed in 25µl aliquots as follows: IL-1β, E2 (PGE2) and PAI-2 were analysed using a commercially available enzyme-linked immunosorbent assay (IL-1β ELISA, Roche, Mannheim, Germany), (E2 (PGE2): R&D Systems, Wiesbaden, Germany), (PAI-2: American Diagnostica, Pflugstadt, Germany). All assays were performed according to the manufacturer's instructions using human recombinant standards.

TNF-α detection was performed using a 10% SDS PAGE and Western blotting analysis, using a human TNF-α standard of known concentration and benchmark protein ladder (Invitrogen GmbH, Karlsruhe, Germany). Following incubation with the primary AB (1:1000) anti-TNF-α IgG (Santa Cruz, California, USA) samples were blocked with TBS buffer pH 7.5 containing 0.1% Tween 20.

Detection occurred by incubation with the second AB (1:2000) anti-goat IgG-AP labeled (Santa Cruz) and NBT/BCIP (Roche) substrate for 2 h RT. The evaluation of the TNF-α amount was performed by comparison of standards and samples. Results are reported as concentrations by converting Periotron units to volume using serum calibration curves as described by Lamster et al. [19]. To prove the

influence of degradation in GCF samples, we compared GCF samples, stored at 4°C and room temperature (RT) for 0 and 4 hours. Standard reagents in BSA were used as a control.

Statistics

The statistical tests were carried out as indicated between GCF / PICF of the studied patient groups. The means for each group were calculated.

All data analyses were performed using JMP statistical software package (SAS Institute Inc.). For plaque statistics all tested sites were considered 100%, infected sites were given in % of total tested sites.

Result

Clinical examination

The two healthy control groups showed adequate oral hygiene and were measured twice within an interval of 6 months to obtain reliable parameters and avoid intra-individual differences. No significant differences were observed between the control groups with respect to mean age and sex distribution (see Material & Methods). The healthy implants showed no signs of inflammation or alveolar bone destruction, the gingival tissue appeared healthy. The mean time since implant loading was within 87 months.

The two disease groups with periodontitis and peri-implantitis were of older age compared to the healthy control groups. The mean time since implant loading was 36 months.

Bacterial flora and pocket depths (PD)

The distribution of five different bacterial species in the sulcus of all study groups showed significant differences.

Marginal amounts of bacteria were seen in the group with healthy teeth, between 0 - 1% and the healthy implant group between 0.5 - 1.5% (Figure 1). In the peri-implantitis group, Porphyromonas gingivalis (P.g) was 100% present at all sites, the other bacterial species were present in 28% of all sites. Periodontitis showed a 100% Porphyromonas gingivalis (P.g) colonisation and 80% of Bacteroides forsythus (B.f) and Treponema denticola (T.d). Actinobacillus actinomycetemcomitans (Haemophilusactinomycetemcomitans) (A.a) and Prevotella intermedia (P.i) were present in 30% of all sites (Figure 1) (BACTERIAL FLORA & PROBING DEPTHS).

Mean mesial probing depths showed a significant increase from healthy teeth to healthy implants to periodontitis with the peri-implantitis group having the greatest pocket depths (Tables 1 and 2).

Group	Mean	Standard error	Lower 95%	Upper 95%
Healthy teeth	2.55	0.27	1.99	3.10
Healthy implants	3.55	0.27	2.99	4.10
Periodontitis	5.50	0.39	4.70	6.29
Peri-implantitis	7.36	0.37	6.60	8.11

Table 1: One-way analysis of mesial probing depths (PD) given in mm.

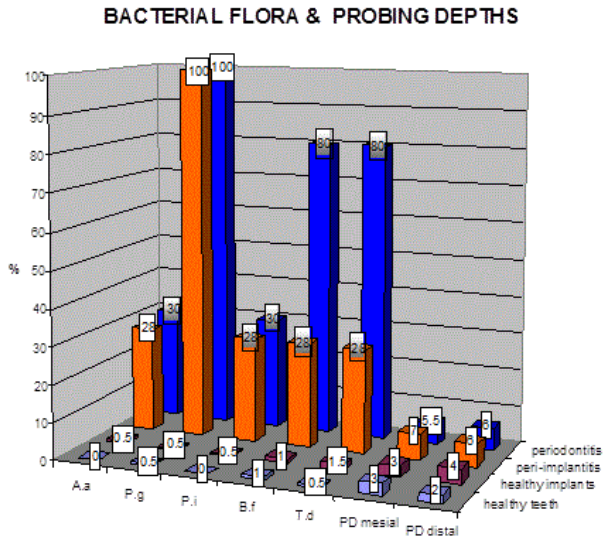

Figure 1: Each tooth/ Impalnts was tested mesial and distal (= 2 sites) and the appearance of the tested bacteria were given in percent to total number of sites. The sensitivity of the used PCR-Test was >104 bacteria; Z-AXIS: Distribution of bacteria in the pockets of: healthy teeth, healthy implants, peri-implantitis and periodontitis. The mean mesial and distal pocket depths (PD) are given in mm; X-AXIS: On the x-axis the different species are given : A.a: *Actinobacillus actinomycetemcomitans* (Haemophilus actinomycetemcomitans); P.g: *Porphyromonas gingivalis;* P.i: *Prevotella intermedia;* B.f: *Bacteroides forsythus* (Tannerella forsythensis); T.d: *Treponema denticola*

The same results were recorded for the distal probing depths where the differences between periodontitis and peri-implantitis were minimal. Mean probing depths were a third deeper in the peri-implantitis group (7.4 - 8 mm) than in the periodontitis group (5.4 - 5.7 mm). Probing depths were on average twice as high in the disease groups as in the healthy control groups.

Group	Mean	Standard error	Lower 95%	Upper 95%
Healthy teeth	2.50	0.22	2.04	2.95
Healthy implants	3.65	0.22	3.19	4.10
Periodontitis	5.80	0.32	5.15	6.44
Peri-implantitis	6.27	0.30	5.65	6.88

Table 2: One-way analysis of distal probing depths (PD) given in mm.

Sulcus fluid component

The GCF / PICF mediator profiles of all groups are given as mean concentrations (ng/ml) shown in Figure 2 (SULCUS FLUID PROFILES).

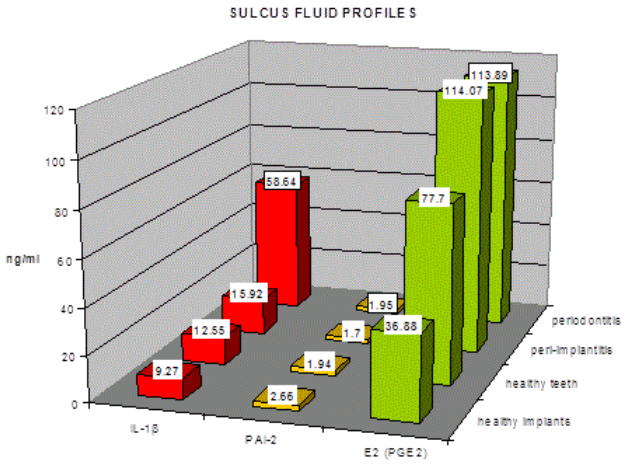

Figure 2: Characteristic GCF/PICF profiles of healthy implants, healthy teeth, peri-implantitis and periodontitis. Mean concentration is given as ng/ml per tooth respectively implants; IL-1β (red), PAI-2 (yellow) and E2 (PGE2) (green).

IL-1ß

In PICF from healthy implants, significant lower IL-1β levels ($\chi i2=0.030$) were found compared to GCF from healthy teeth. Peri-implantitis and periodontitis sites revealed a significant increase of IL-1β levels ($\chi i2= 0.0015$). The periodontitis group showed the highest IL-1β concentration in the sulci of all groups (Table 3 and Figure 2).

Group	Mean	Standard error	Lower 95%	Upper 95%
Healthy teeth	12.55	6.10	0.26	24.83
Healthy implants	9.27	4.31	0.58	17.96
Periodontitis	58.64	6.10	46.35	70.92
Peri-implantitis	15.92	5.82	4.20	27.63

Table 3: one-way analysis of IL-1β given in ng/ml.

E2 (PGE2)

The E2 (PGE2) level in PICF of healthy implants was significantly lower ($\chi i2=0.040$) than in the GCF of healthy teeth.

Group	Mean	Standard error	Lower 95%	Upper 95%
Healthy teeth	77.70	26.03	25.33	130.08
Healthy implants	36.88	18.41	-0.15	73.92
Periodontitis	113.89	26.03	61.51	166.27
Peri-implantitis	114.07	24.82	64.14	164.01

Table 4: one-way analysis of E2 (PGE2) given in ng/ml.

High levels of E2 (PGE2) concentration were found in the peri-implantitis as well as in the periodontitis groups (Table 4 and Figure 2).

PAI-2

The levels of PAI-2 in all four groups were virtually low. Highest level in the healthy implants group, lowest level in the peri-implantitis group. For healthy teeth and periodontits we found about equal values (Table 5, Figure 2).

Group	Mean	Standard error	Lower 95%	Upper 95%
Healthy teeth	1.94	0.56	0.79	3.08
Healthy implants	2.66	0.40	1.85	3.47
Periodontitis	1.95	0.56	0.80	3.09
Peri-implantitis	1.70	0.54	0.61	2.79

Table 5: one-way analysis of PAI-2 given in ng/ml.

TNF-α

TNF-α was very low but measurable, showing highest levels in the peri-implantitis group, lowest in the healthy teeth group (Table 6).

Group	Mean	Standard error	Lower 95%	Upper 95%
Healthy teeth	0.70	0.22	0.25	1.14
Healthy implants	1.10	0.22	0.66	1.54
Periodontitis	0.90	0.31	0.27	1.52
Peri-implantitis	1.54	0.29	0.94	2.14

Table 6: one-way analysis of TNF-α levels given in ng/ml.

Discussion

Mediator levels within GCF / PICF have been considered as markers of the progression and severity of periodontal disease as well as response to the individual host immune system. We selected from a variety of bone-resorbing and potentially protective components four markers benefical for monitoring periodontal health status and evaluated quantitative levels of IL-1β, E2 (PGE2), PAI-2 and TNF-α within healthy teeth, healthy implants, periodontitis and peri-implantitis groups. Probing depths and bacterial colonisation were additionally examined. Results were expressed as the average of all sampled sites within each group. The sampling period was extended to six months to avoid any differences due to circadian influences. All patients showed measurable levels of all four markers.

Healthy teeth and healthy implants

Within all groups tested, we found the lowest levels of IL-1β and E2 (PGE2) in the healthy implants group. Surprisingly the healthy implants group showed at the same time the highest level of PAI-2.

In the healthy teeth group the level of IL-1β was higher than in healthy implants and E2 (PGE2) was twice as high as for healthy implants. PAI-2 showed a lower level for healthy teeth than for healthy implants.

We hypothesize that the lower levels of E2 (PGE2) and IL-1β in the sulcus of healthy implants could be – among other factors - due to the lack of PDL-cells with their ability of producing exudates enriched with inflammatory cytokines. Inflammatory processes caused by microorganisms may constantly take place around teeth resulting in elevated E2 (PGE2) and IL-1β production by intact PDL cells. During inflammation IL-1β may modulate PDL cell functions, allowing PDL cells to participate directly in the disease process [20]. Implants have no active "defense mechanism" from PDL cells which could explain low E2 (PGE2) and IL-1β sulcus concentrations. Another reason for low levels of E2 (PGE2) and IL-1β in the healthy implants group could also be the anti-inflammatory effect of titanium surfaces of implants [21,22].

Bacterial colonization was at low level both for the healthy teeth and healthy implants groups, lowest in healthy teeth. Mean probing depths for healthy teeth were ≤ 3 mm, for healthy implants ≥ 3 mm.

PAI-2 is an inhibitor of the protease plasmin activation [6]. Plasminogen activator t-PA and PAI-2 are found at high concentrations especially in areas of gingival inflammation [23]. It is described that the state of inflammation is expressed in high or low PAI-2 levels influenced by inflammatory mediators [17,24]. Other authors suggest that PAI-2 could be involved in wound healing after periodontal therapy [25]. In this study the highest PAI-2 value was found in the healthy implants group [26].

TNF-α is supposed to be another pro-inflammatory cytokine with many functions [27,28]. TNF-α stimulates bone resorption and fibroblasts to produce collagenase [29,30]. The detected levels of TNF-α in this study were very low for all four groups. The lowest level for TNF-α was found in the healthy teeth group.

Periodontitis and peri-implantitis

Interleukin IL-1β and prostaglandin E2 (PGE2) are known to play a central role in host response as mediators of local tissue destruction and bone resorption [31,32]. In an earlier study it was reported that IL 1 can regulate its own production through a self-induced inhibitor E2 (PGE2) [33].

Significant differences between the periodontitis and peri-implantitis groups were detected. We found significantly higher levels of IL-1β in periodontitis than in peri-implantitis. Earlier studies comparing simultaneously periodontitis and peri-implantitis for clinical signs and levels of IL-1β and E2 (PGE2) could not be found. In line with our results for periodontitis patients higher amounts of IL-1β and E2 (PGE) were reported [34-38]. For peri-implantitis patients another study found higher levels of IL-1β modulating attachment loss in implants [39].

In our data we found no significant difference in E2 (PGE2) levels between the periodontitis and the peri-implantitis groups. Both showed significantly high levels compared to healthy controls. These results could confirm previous findings of associations between clinical signs of periodontitis and IL-1β and E2 (PGE2) [37]. The bacterial load in the sulcus of the periodontitis and the peri-implantitis groups showed a significantly higher value compared with healthy controls. The highest bacterial prevalence was observed in the periodontitis group. Mean probing depths for periodontitis were ≥ 5 mm, for peri-implantitis ≥ 6 mm.

The lowest PAI-2 levels were found in our peri-implantitis group. A study on periodontitis patients with type 2 diabetes mellitus reported high levels of PAI-2 and low levels of IL-1β reflecting a depressed immunological answer [26]. As above mentioned, in our study patients with type 2 diabetes mellitus were excluded. We found for our periodontitis patients significantly high levels of IL-1β and mean levels of PAI-2. In earlier studies of periodontitis the production of PAI-2 seemed to be also locally lowered by impaired sites [17]. In all tested groups only small variations in the amounts of PAI-2 were observed. These results are in accordance with earlier studies for gingivitis and periodontitis sites [6].

To place this study in context, our results indicate that elevated IL-1ß and E2 (PGE2) levels together with high bacterial colonisation and deeper probing depths may be useful markers of ongoing periodontal inflammation.

The mean concentration of TNF-α was very low but measurable showing the highest level in the peri-implantitis group. The second highest value was recorded for healthy implants, followed by periodontitis and was lowest for healthy teeth. Because of very low levels, these results should be interpreted with caution. It is also reported that TNF-α is more present in active lesions [40,41]. In another study TNF-α was detected in 37 of 42 implant samples, but no correlations between the severity of peri-implant inflammation and the presence of TNF-α were found [13]. According to other findings it remains unclear whether the level of TNF-α in PICF and CGF is a marker for current periodontal status [42].

Conclusions

Within the limits of this study we reached the following conclusions:

The inflammatory agents IL-1β and E2 (PGE2) are lowest in the healthy implant group, rise in the healthy teeth group, worsen in the peri-implantitis group and peak in the periodontitis group. The healthiest non inflammatory sulcus fluid may be associated with healthy implants and least with periodontitis. This confirms observations made by implantologists that implants in patients with periodontitis last longer than the patients own teeth. The anti-inflammatory property of titanium surfaces of implants could possibly contribute to this effect. Host-related intrinsic and induced factors and genetic disposition may also be of great importance. To better understand what precise molecular / cellular mechanisms potentially influence above findings, a larger prospective study with long term evaluation would be required.

Conflict of Interest

The authors declare that they have no conflict of interest.

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