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Dentistry Section

Peri-Implant Sulcus Fluid (PISF) Matrix Metalloproteinase (MMP) -8 Levels in Peri-Implantitis

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ABSTRACT

Introduction: Matrix Metalloproteinase (MMP) -8 plays crucial role in pathogenesis of periodontitis and is also a possible biomarker candidate in peri-implantitis.

Aim: The aim of the study was to analyse MMP-8 levels in peri-Implant Sulcus Fluid (PISF) from peri-implantitis affected implants in smoking and non-smoking patients with different periodontal health status of natural teeth before and after peri-implantitis treatment.

Settings and Design: Altogether 29 patients with peri-implantitis were recruited and divided into two study groups (11 with healthy periodontium or gingivitis, i.e. no marginal bone loss, and 18 with chronic periodontitis).

Materials and Methods: PISF sample from one implant with peri-implantitis from each patient was collected at the baseline and six months after conservative and surgical peri-implantitis treatment, and clinical parameters were registered. Samples were analysed for MMP-8 with dento ELISA method applying a monoclonal antibody. Mucosal cell samples were also analysed for IL-1 gene polymorphism. PISF MMP-8 levels' differences between periodontal diagnosis groups and between smokers' and non-smokers' were analysed. Also, IL-1 polymorphism profiles were compared between study groups.

Results: PISF MMP-8 levels were higher at the baseline compared to and after the treatment when all sampled implant sites were analysed together ($p = 0.001$). MMP-8 levels' distribution was broader in periodontitis patients' PISF samples, and only in periodontitis patients' group levels decreased statistically significantly after the treatment ($p = 0.005$). Smokers'and non-smokers' PISF MMP-8 was at similar level both at the baseline and after the treatment. No difference between distributions of IL-1 genotypes was found between study groups.

Conclusion: MMP-8 levels increase in peri-implantitis affected implants both in non-periodontitis and periodontitis patients, but levels still after treatment of the condition reflect intensified host response around implants and indicate challenges of controlling peri-Implantitis with any treatment modality.

Keywords: Host response, Oral implants, Periodontitis, Point-of-care diagnostics

INTRODUCTION

Implants have been used for replacement of lost natural teeth successfully both in periodontally healthy individuals as well as in patients with history of periodontitis [1]. However, in both cases Peri-Implantitis (PI) can develop as a biologic complication if health of mucosal structures surrounding implants cannot be optimally maintained. Based on several studies, periodontitis patients may be more at risk of PI than periodontally healthy [2-5]. Smoking is also regarded as a risk factor for biologic implant complications [4,6].

Despite in many aspects similar etiologic factors and pathogenesis of periodontitis and PI, a crucial difference may be the structure of implant's surrounding tissues compared with periodontium of natural teeth. Implant surface lacks the cementum and gingival collagen fibers attachment apical to the junctional epithelium is also missing. Furthermore, because no periodontal ligament exists, blood flow is less effective in implant surrounding tissues than in the periodontium of natural teeth. These conditions are regarded to be decisive for the control of host response in PI [7]. If PI with loss of the surrounding bone of the titanium implant fixture develops, offering a connection from oral cavity environment to the rough implant surface, progression of bone loss is fast compared to periodontitis, controlling of the situation is demanding and the response to treatment procedures is unpredictable [8,9].

Matrix Metalloproteinases (MMPs) play a crucial role in various tissue destructive inflammatory processes by degrading almost all peri-cellular and basement membrane components, and MMP-8 is known to be the major MMP in periodontitis [10-12]. For that reason MMP-8 may be a possible candidate as an adjunctive diagnostic biomarker in peri-implant diagnostics as well. PISF may offer similar possibilities in diagnosing the level of inflammation and markers of tissue destruction around implants as Gingival Crevicular Fluid (GCF) in natural teeth. PI PISF is known to contain higher MMP-8 levels and activity than GCF from chronic periodontitis sites with similar depth, and PI PISF also exhibits high activation of MMP-8 isoenzyme species (PMN and fibroblast-type) [11-13]. In a recent study also Arakawa et al., detected MMP-8 from PI PISF by western blotting, and MMP-8 was the only detected collagenase [14]. Polymorphism in promoter region of MMP-8 gene may be associated with early implant failures [15]. PISF MMP-8 levels have been demonstrated to have positive correlations with gingival index and probing depth in loaded implants [16].

Interestingly, PI can also affect periodontally healthy individuals, i.e. irreversible connective tissue destruction around implant in an individual who is not periodontitis affected is not unusual. For this reason we examined the matrix metalloproteinase (MMP) -8 response in PI PISF both in periodontally healthy or gingivitis patients and untreated periodontitis patients with PI, and compared the levels after the active treatment phase.

Our hypothesis was that monitoring of MMP-8 level as single biomarker from PISF gives information about peri-implant tissue health and disease, and that MMP-8 is possible candidate as a biomarker with clinical diagnostic utility in implantology.

MATERIALS AND METHODS

Study population

Study population comprised 29 partially dentate patients. Sample size was decided after consulting a biostatistician. Inclusion criteria for participation in this study were the presence of at least one bone level screw implant with PI and satisfactory cemented implant restoration where the possibility of residual subgingival cement

was excluded by radiographs, no occlusal overload, good oral hygiene as evidenced by a plaque control record < 30% after oral hygiene instructions, no systemic diseases, no bisphosphonate or tetracycline/doxycycline treatment, negative culture bacterial analysis results for *Aggregatibacter actinomycetemcomitans* and no periodontal treatment before and after implantation. For bacterial analysis (performed by ParoCheck20, GreinerBioOne) samples were obtained from each study participant from five deepest periodontal pockets with paper points. Sampling method is described in detail by Thierbach & Eger [17].

Patients' status of natural teeth varied from periodontal health (N=8) or gingivitis (no alveolar bone loss, bleeding on probing > 20%; N = 3) to different degrees of chronic periodontitis (N = 18). Patients for the study were recruited among employees of German army between February and May 2010, and the study was completed in January 2011. Patients were examined and treated at German armed forced central hospital – Dept. VIIa- Periodontology by one calibrated periodontologist (RT). The study protocol included detailed oral and radiographic examinations, PISF sampling from one selected implant site with PI at the baseline and after the treatment, and mucosal cell sample for interleukin (IL) -1 genetic testing at the baseline. The investigation conforms to the principles of the Declaration of Helsinki of 1975, revised 1983, and was approved by the German army ethics committee. All patients gave written consent for participation.

Periodontal/peri-implant diagnosis and treatment protocol

At baseline, all patients went through a thorough periodontal examination including pocket Probing Depths (PD), Clinical Attachment Level (CAL) and Bleeding On Probing (BOP) from 6 surfaces on each tooth/implant. The degree of alveolar bone loss of natural teeth was evaluated from panoramic dental radiographs (Sirona, OrthophosXGplus, Bensheim, Germany) and of implants with single-tooth intra-oral parallel, right-angle technique radiographs (Sirona, Heliodent DS, Bensheim, Germany) of the implant region comprising total implant length. All radiographs were viewed in a darkened room using a radiograph screen (Schulte, Planilux, Warstein, Germany). Bone loss from the most apical part of the bony defect to the apical margin of the abutment in percent and millimeter was assessed from most affected implant site of each patient. For the percentage measurement Schei ruler was used. One examiner performed all radiographic assessments. Criteria for PI included PD > 5 mm and bone loss > 3 mm as evidenced by clinical and radiographic examinations, and bleeding on probing and/or suppuration. The position of the implants after the insertion was evaluated from the intraoral radiographs, and no study implant was inserted in a subcrestal position. Criteria for periodontal diagnosis followed the criteria of the American Academy of Periodontology.

At the baseline (BL), supragingival plaque was removed in all patients. Fourteen days later, subgingival debridement (full-mouth scaling and root planing) of all pathologically deepened pockets was performed under local anaesthesia in all four quadrants of all patients in accordance with the principles of full-mouth disinfection with ultrasonic- (SonicFlex, KaVo, Biberach, Germany) and hand-instruments and application of chlorhexidinedigluconate (0.12%) (Paroex, Sunstar, Kriftel, Germany). Adjuvant antibiotic treatment with metronidazole 400 mg (1-1-1) was performed for ten days in patients in whom the presence of anaerobic bacteria (> 10%) was detected (N = 24). All patients underwent antimicrobial Photo Dynamic Therapy (aPDT) using a Low-Intensity Laser Treatment (LILT) laser (TheraLite laser, HelboPhotodynamics Systems, Grieskirchen, Germany) of the implant pockets. For this purpose, a photosensitizer (phenothiazine chloride, Helbo®Blue, HelboPhotodynamics Systems, Grieskirchen, Germany) was inserted into all pathologically deepened implant-pockets and removed with saline solution after three minutes. The defects were then exposed to laser light with a wavelength of 660 nm for ten seconds using fiber optics. Light was delivered to six sites per implant. Four months later the patients underwent access flap surgery of the PI sites included in the study. The clinical treatment result was evaluated six months after the flap surgery.

PISF sampling and processing

PISF samples for MMP-8 analysis were collected from one implant site per patient, which was the most severe PI lesion site at the baseline, before the baseline examination and at the final examination when the treatment result was evaluated six months after the flap surgery (later in text mentioned as the six months evaluation). For this purpose, the site to be sampled was isolated from saliva contamination, cleaned of supragingival plaque, rinsed and dried. PISF samples were then collected with paper strips which were inserted into the sulcus for ten seconds avoiding gingival bleeding. The samples were placed in special transportation tubes and stored at -20°C degrees until thawed for analyses. MMP-8 analysis was performed by dentoELISA immunoassay (Dentognostics, Jena, Germany) according to Leppilahti et al., [18].

IL-1 genotyping

Epithelial cells were obtained from the buccal mucosa with sterile swabs [19]. Samples were analysed for IL-1 polymorphisms. PCR was used to detect polymorphisms at the IL-1A +4845 and IL-1B +3954 loci of the IL-1 gene cluster according to manufacturer's instructions (HainLifeScience, Nehren, Germany).

STATISTICAL ANALYSIS

Statistical analysis were performed with PASW Statistics 18 (Statistical Package for the Social Sciences). For statistical analysis the patients were grouped based on periodontal disease status of the natural teeth into patients with healthy periodontium or gingivitis (N = 11), i.e. no marginal bone loss, and patients with chronic periodontitis (N = 18), and according to smoking status. The differences between the characteristics of the study groups were analysed by the t-test, the Mann-Whitney test, and the Pearson Chi-Square test. MMP-8 levels were expressed as mean levels with 95% confidence interval (95% CI), and the comparisons were made by using non-parametric tests: differences between smokers' and non-smokers' PISF MMP-8 levels and between periodontal diagnosis groups were tested with Mann-Whitney test, and the difference between baseline and post treatment MMP-8 levels with Wilcoxon test.

RESULTS

[Table/Fig-1] displays the characteristics of periodontally healthy/gingivitis (no marginal bone loss) and periodontitis groups. Number of female patients was four out of total 29; all of them belonged to periodontitis group. Of characteristics, total PD value (mean value for all PD measurements both for natural teeth and implants) and BOP percentage differed statistically significantly (p = 0.044 and 0.024 respectively) between the study groups.

At the baseline mean (95% CI) MMP-8 level for all sampled implant sites (N = 29) was 96.4 (49.0-143.9) ng/mL and at the six months evaluation after the treatment phase14.4 (6.6-22.3) ng/mL (p = 0.001) [Table/Fig-2,3a]. When non-smokers and smokers were analysed separately [Table/Fig-4], in both subgroups of implant sites PISF MMP-8 levels decreased statistically significantly after the treatment (p = 0.011 and 0.037 respectively). No difference between non-smokers' and smokers' PISF levels was found neither at the baseline nor at the six months measurement.

In periodontitis patients' group PISF MMP-8 levels were at six months evaluation significantly lower compared with the baseline

	Healthy/gingivitis N = 11	Periodontitis N = 18	p-value
Gender (male) n (%)	11 (100)	14(77.8)	0.092*
Age mean (SD)	55.5 (9.8)	56.4 (8.2)	0.088†
Smokers n (%)	5 (45.5)	12 (66.7)	0.260*
Pack years mean (SD)	7.0 (7.8)	6.1 (10.5)	0.816†
PD total mean (SD)	2.9 (0.4)	3.5 (0.8)	**0.044‡**
BOP total mean (SD)	15.8 (12.3)	28.3 (14.8)	**0.024‡**
Age of implant mean (SD)	5.4 (3.2)	7.2 (4.0)	0.371‡
IL-1 risk type			0.320*
A	3 (27.3)	2 (11.1)	
B	1 (9.1)	7 (38.9)	
C	5 (45.5)	6 (33.3)	
D	2 (18.2)	3 (16.7)	

[Table/Fig-1]: Patient characteristics (N = 29).
Significant p-values indicated as bolded
* Pearson Chi-Square test
†t-test
‡Mann-Whitney test

levels (p = 0.005) [Table/Fig-3b], and also when non-smoking and smoking patients were analysed separately (p = 0.043, p = 0.047 respectively) [Table/Fig-5]. In control group no significant difference between the baseline and the six months evaluation MMP-8 levels was detected [Table/Fig-3b]. Finding was similar when smoking and non-smoking periodontally healthy/gingivitis subjects were analysed separately [Table/Fig-5]. Periodontitis patients' mean PISF MMP-8 level decreased to less than seventh part between the baseline and the six months evaluation (from 130.7 to 17.3ng/mL), while in periodontally healthy/gingivitis subjects' group mean MMP-8 level reduced to fourth part (from 40.3 to 10.2ng/mL). In periodontally healthy/gingivitis subjects' group the baseline MMP-8 distribution was substantially narrower than in periodontitis group showing that MMP-8 levels in periodontitis patients' PISF may be significantly higher than in periodontally healthy/gingivitis subjects, and after the treatment distributions were nearly similar [Table/Fig-3b].

Mean (SD) PD for study implants was 3.5 (0.9) mm at the baseline and 2.7 (0.8) mm at the six months evaluation; maximum PD values for study implants were 7.9 (1.1) mm and 4.8 (0.9) mm, respectively. Mean (SD) CAL for study implants was 5.2 (1.0) mm at baseline and 3.5 (0.7) mm at the six months evaluation. Mean BOP for study implants was 76% at baseline and 12% at the six months evaluation.

Risk alleles for IL-1 polymorphisms were present in 53.57% of the patients. A defective allele for the IL-1 receptor antagonist (IL-1ra) was detected in 39.29% of the patients. However, no significant difference between distributions of IL-1 genotypes was found between study groups [Table/Fig-1].

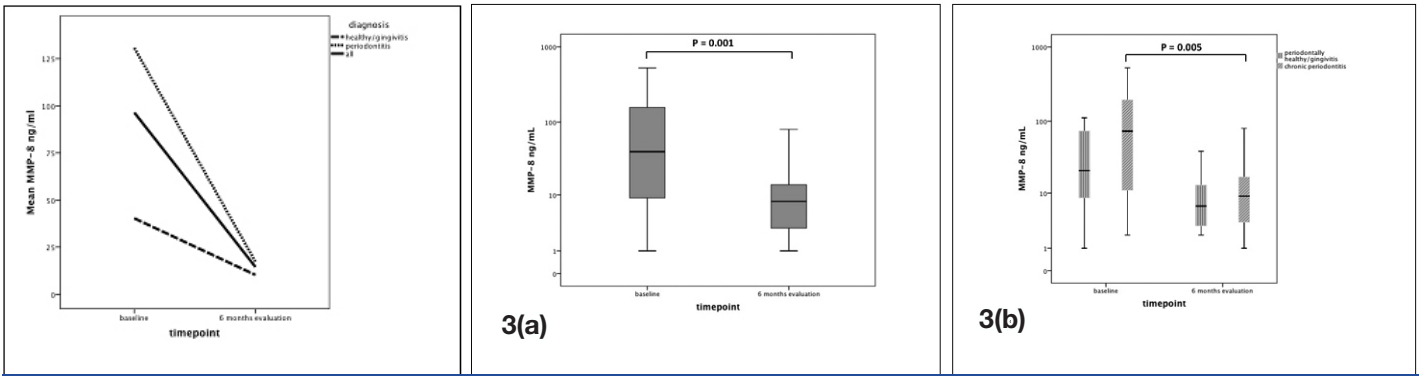
DISCUSSION

Peri-implantitis is a biologic implant complication seriously compromising prognosis of affected implants. Arresting this condition with any treatment is still empirical and based on methods designed for individual cases [20]. Diagnostic aid for early detection of the complication and means for follow-up during the maintenance and after PI treatment to avoid further problems would be valuable for clinicians.

Our findings regarding PISF MMP-8 analysis supplemented and further extended the previous studies revealing that MMP-8 in PISF can be regarded as a potential biomarker of PI similarly as Gingival Crevicular Fluid (GCF) MMP-8 assessment in periodontitis [10,13,14,16,21,22]. For analyses we pooled together periodontally healthy subjects and gingivitis patients (subjects with no marginal bone loss) and compared their PI PISF MMP-8 sample levels with periodontitis patients' levels, based on the background knowledge that periodontitis patients may be particularly at risk of PI [2-5]. We found that increased PISF MMP-8 levels from PI-affected implants were found both from patients with periodontitis and those who were non-periodontitis subjects, but the distribution of MMP-8 levels was broader in periodontitis patients' PISF, i.e. PI patients with coexisting periodontitis may have higher MMP-8 PISF levels than patients who represent no irreversible changes in their natural teeth attaching structures. Conservative and surgical treatment of PI resulted in statistically significant reduction of PISF MMP-8 levels, and especially in periodontitis group. However, still after the treatments PISF MMP-8 levels remained at moderate levels indicating possible difficulties to get the situation in control.

Endogenic proteolytic enzymes participate in normal tissue regeneration including bone resorption and formation. MMPs are necessary for maintaining normal tissue homeostasis, they have a role in wound healing, in immunity and in several diseases [23]. MMPs activate other pro-inflammatory transmitters, and especially MMP-8 is an important mediator of LPS induced inflammation. Choosing MMP-8 as the biomarker of special interest of our study was based on the knowledge received from numerous studies concerning its role in inflammatory cascade [23] and its potential role as chair-side diagnostics tool of periodontal disease status around natural teeth when analysed from oral fluids (gingival crevicular fluid, oral rinse sample, saliva) [18,24]. For clinical use in implant patient care it would be convenient to have a test for diagnostic purposes, and interpretation of the test result if it is based on one biomarker would be practical and convenient.

Collagenase-2 (MMP-8) has been found to be pathologically elevated and converted to active form in PI-affected PISF and accordingly differing from MMP-8 detected in PISF from peri-implant mucositis-affected and healthy oral implants [11,12,16]. These MMP-8 findings correspond to those observed in periodontitis GCF versus gingivitis and healthy GCF [25-27]. In this regard peri-implant soft tissues have been demonstrated to develop



[Table/Fig-2]: Change of MMP-8 mean levels between baseline and six months evaluation in all studied implant sites and in implant sites from study subjects with different periodontal disease status. **[Table/Fig-3a,b]:** Box-and-whiskers plots indicating PISF MMP-8 medians and quartiles at baseline and at six months evaluation: a) for all studied implant sites; and b) for implant sites in study subjects with different periodontal disease status with p-values for statistically significant differences.

	Non-smokers (n=17) MMP-8 ng/mL	Smokers (n=12) MMP-8 ng/mL	p-value*
Baseline	111.9 (34.2-189.7)	74.4 (27.5-121.3)	0.811
6 months	10.4 (2.2-18.7)	19.6 (3.9-35.2)	0.609
p-value†	**0.011**	**0.037**	

[Table/Fig-4]: Smokers' and non-smokers' baseline and six months evaluation visit mean (95% CI) MMP-8 levels from studied implant sites with peri-implantitis, one sampled site per patient.
Significant p-values indicated as bolded
*Mann-Whitney test between smoking and non-smoking periodontally healthy/gingivitis subjects' and periodontitis patients' implants
†Wilcoxon test between baseline and post treatment measures

	MMP-8 ng/mL mean(95% CI)		
Periodontal diagnosis	baseline	6 months	p-value†
Healthy / gingivitis (N=11)	40.3 (12.7-67.9)	10.2 (2.3-18.2)	0.086
Smoker (N=6)	40.2 (-11.7-92.1)	13.3 (-0.7-27.3)	0.345
Non-smoker (N=5)	40.4 (-2-82.8)	5.5 (-2.6-13.6)	0.144
p-value*	0.931	0.257	
Periodontitis (N=18)	130.7 (58.0-203.4)	17.3 (4.7-29.8)	**0.005**
Smoker (N=6)	108.7 (22.6-194.8)	27.0 (-12.4-66.4)	**0.043**
Non-smoker (N=12)	141.8 (32.5-251.0)	12.4 (0.62-24.2)	**0.047**
p-value*	1.0	0.371	

[Table/Fig-5]: MMP-8 mean (95% CI) levels of implants with peri-implantitis in smoking and non-smoking patients grouped by periodontal status of natural teeth at baseline and at six months evaluation.
Significant p-values indicated as bolded
*Mann-Whitney test between smoking and non-smoking periodontally healthy/gingivitis subjects' and periodontitis patients' implants
†Wilcoxon test between baseline and post treatment measures

a stronger inflammatory response to plaque accumulation in relation to their gingival counterparts when assessed by potential biomarker including MMP-8 [28]. Additionally, a cause-and-effect relationship has been demonstrated between biofilm formation and development of both gingivitis and peri-implant mucositis, which were found to be reversible by addressing GCF and PISF biomarker MMP-8 [28]. In periodontitis-affected GCF repeatedly elevated and activated MMP-8 reflects and predicts periodontal disease progression [29-32], and successful periodontal treatment decreasing elevated and activated GCF MMP-8 reflects periodontal health and reduced risk of disease progression [30,32,33]. Recent evaluation revealed in a prospective study that PISF MMP-8 assessment was useful in monitoring the course of peri-implant disease, and MMP-8 was found to be an early sign of peri-implant inflammation [16].

In a recent cross-sectional study [34] where several biomarkers (IL-1β, VEGF, MMP-8, TIMP-2, and OPG) together with periodontopathogenic bacteria (*A. actinomycetemcomitans, P. intermedia, P. gingivalis, T. forsythia, and T. denticola*) were analysed, MMP-8 did not reveal meaningful difference among groups. Healthy and PI-affected implants were not compared based on the status of natural teeth and no treatment was given, which makes it difficult to compare the result with ours. In our study PI-affected implants were also treated with flap surgery. In a study where PI was treated with surgery and PISF levels of bone markers were analysed, a significant reduction of MMP-8 together with interleukin-6 and bone resorption markers were detected being in line with our finding [35].

In periodontitis patients' GCF MMP-8 levels may vary strongly, and in data analyses this is manifested as broad distributions [30,32]. Especially high levels can be regarded as a sign of an enhanced site level host response and also as a possible indication of patient level host response: not only sites with bone loss and deep periodontal pockets, but also periodontitis patients' periodontally healthy sites may have significantly high GCF MMP-8 levels, and even successful treatment does not lead to GCF MMP-8 levels comparable with periodontally healthy subjects' sites [27]. According to the result of the current study, the individual level of host response is reflected also in PISF from PI-affected implants. However, also patients with no marginal bone loss in their natural teeth had high MMP-8 levels in their PI-affected implants PISF showing an increased local host response. Thus it can be interpreted that rough implant fixture surface, when exposed to oral bacteria, creates an excessive host response as well in periodontitis patients as in non-periodontitis subjects and can possibly be explained as a foreign body reaction.

Smoking has been reported to both decrease and increase GCF MMP-8 levels [30,32,36]. Thus, smoking may be a significant confounding factor also in PISF MMP-8 analyses, but our study did not show any remarkable effect of smoking on PISF MMP-8 levels. The possible explanation also for this may be the more intensive host response of PI affected implants because of exposed rough titanium surface transformed as foreign object, compared to natural teeth root surface, which may override the effect of smoking. This aspect needs further studies with larger patient sample.

We were aware when designing the study, that analysing of one biomarker may not be diagnostically sufficient. However, MMP-8 reflects the first line of innate immune response, and based on our results MMP-8 is a potential biomarker to be used in conjunction with clinical parameters for monitoring peri-implant health and disease. A possible strategy would be to analyse MMP-8 simultaneously with bone loss biomarkers like soluble RANK ligand (sRANKL), osteoprotegerin (OPG) and sclerostin which have been found to be significantly increased in patients with PI compared with patients healthy peri-implant tissues, and suggested as prognostic biomarkers in PI [37]. In a recent review interleukin-1β was added into the list with the aforementioned markers of promising candidates in differentiating PI from implant health, but also the need for further studies was recognised [38]. Detection of MMP-8 together with prostaglandin E2 was noticed useful in a longitudinal study in which oral implant health was longitudinally monitored after implantation for 18 months [16].

MMP-8 can especially be used as an indicator of enhanced host response. Our dento ELISA-assay utilizes an MMP-8 antibody that is selective for active form of MMP-8 [31,39]. Active form of MMP-8 in GCF is characteristic of active periodontitis lesions, and in PISF it may also be characteristic of active PI lesions/sites [11,12,16,25,26,29]. Our sample size was small and another possible weakness of the study is that the majority of study subjects were male. Also, our control group consisted of implants from non-periodontitis subjects with peri-implantitis. Study with similar setting should be repeated with a larger study population and with separate groups of PI patients with periodontal health, gingivitis and periodontitis of natural teeth.

CONCLUSION

As conclusion, MMP-8 levels are increased in PI affected implants both in non-periodotitis and periodontitis patients, and the levels decrease by treatment. However, still after the treatment of PI, PISF MMP-8 levels reflect an intensified innate host response and indicate the challenges of controlling PI with any treatment modality. Combining MMP-8 with bone resorption markers would possibly be recommendable.

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CONFLICT-OF-INTEREST

Kurt Maier is the CEO of Dentognostics; Dr. Sorsa is an inventor of oral fluid diagnostic US patents (5652247, 57336341, 5866432, 6143476). The authors report no conflicts of interest related to this study.

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