Expression of Interleukin-17, Tumor Necrosis Factor-Alpha, and Matrix Metalloproteinase-8 in Patients with Chronic Peri-Implant Mucositis

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEFG 1 **Darius Gleiznys**
BCDE 1 **Albertas Kriauciunas**
AB 1 **Julius Maminskas**
FG 2 **Arturas Stumbras**
CD 3 **Dalia Giedrimiene**
CDE 4 **Christine Niekrash**
AD 1 **Alvydas Gleiznys**
ABEF 1 **Jurgina Sakalauskiene**
ACDE 5 **Astra Vitkauskiene**

1 Department of Dental and Maxillofacial Orthopedics, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania
2 Department of Maxillofacial Surgery, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania
3 School of Health and Natural Sciences and School of Pharmacy, University of Saint Joseph, West Hartford CT, USA
4 Frank H Netter MD School of Medicine Quinnipiac University, North Haven, CT, USA
5 Department of Laboratory Medicine, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

Corresponding Author: Darius Gleiznys, e-mail: darius.gleiznys@lsmuni.lt
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Background: The present study aimed to evaluate whether non-surgical treatment interferes with clinical parameters and local patterns of osteo-immunoinflammatory mediators (IL-17 and TNF-α) and matrix metalloproteinase-8 (MMP-8) that are found in peri-implant crevicular fluid (PICF) and biofilms during the progression of peri-implant mucositis.

Material/Methods: We selected 30 patients with peri-implant caused mucositis before (MP) and after treatment (TP) and 30 healthy people (HP) for the analysis of IL-17, TNF-α cytokine, and MMP-8 production in PICF and for analysis of colonization dynamics of periodontopathogenic bacteria in supra- and subgingival plaque samples. The levels of IL-17 and MMP-8 concentrations in samples were assayed by enzymatic immunosorbent assay (ELISA) and TNF-α levels were determined by enzyme amplified sensitivity immunoassay (EASIA) method in PICF. The micro-IDent test was used to detect 11 species of periodontopathogenic bacteria in subgingival biofilm.

Results: We found significantly ($P<0.001$) higher levels of IL-17, TNF-α, and MMP-8 in the PICF of the MP and TP groups in comparison to the HP group. A significant association was found in MP associated with *Parvimonas micra*, as TNF-α in PICF was significantly higher ($P=0.034$) than in patients without *Parvimonas micra*. TNF-α levels in the samples of PICF showed a moderate correlation with clinical parameters, including plaque index (PI) ($P=0.007$) and MMP-8 levels ($P=0.001$), in the MP group.

Conclusions: Assessment of levels of inflammatory cytokines in PICF can aid in the identification of peri-implant mucositis, which can assist in early diagnosis, prevention, and treatment.

Keywords: Chronic Periodontitis • Interleukin-17 • MMP8 Protein, Human • Tumor Necrosis Factor Alpha (36-68)

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Background

Dental implants can be used for rehabilitation of totally or partially edentulous patients. Utilizing them helps reduce speech discomfort and improve aesthetics and masticatory problems caused by conventional prostheses [1].

Peri-implant mucositis (MP) is defined as inflammation restricted to damage in the soft tissues, which causes bleeding on probing, in comparison with peri-implantitis, which also results in 1 loss marginally that can lead to loss of osseointegration in the future [2]. Bacterial plaque is considered the primary etiological factor of inflammatory peri-implant diseases. In the beginning of these diseases, a cytokine-related response to bacterial products can be observed, such as lipopolysaccharides and endotoxins [3]. Host cells respond to bacterial challenge by releasing certain pro- and anti-inflammatory mediators, which in turn mediate degradation of collagen and extracellular matrix and bone resorption. The release of proinflammatory cytokines and chemokines, such as interleukin -1β (IL-1β), IL-6, IL-8, and tumor necrosis factor-alpha (TNF-α), is strongly associated with disease progression due to their ability to cause bone resorption [4]. Polymorphonuclear cells and an abundance of fibroblast matrix metalloproteinase-8 (MMP-8) have also been shown to be present in the first stages of peri-implantitis, which usually follows the release of these cytokines. The destruction of connective tissue is a significant determinant of the progression of peri-implant lesions and it is essentially driven by MMP-8 [5].

Microorganisms that cause peri-implantitis are similar to bacteria found in periodontitis and gingivitis, such as the species of the red and orange complexes [6]. *Porphyromonas gingivalis, Tannerella forsythia,* and *Prevotella intermedia* are commonly associated with periodontitis, but may not necessarily be correlated with implant status [7].

Recently, Th17-related cytokines were found to be involved in the periodontitis pathogenic process due to their capacity to induce osteoclastogenesis [8]. Th17 cells are characterized by the production of IL-17, which mediates several biological functions associated with the proinflammatory response, including neutrophil and macrophage recruitment, stimulation of proinflammatory cytokine synthesis, and production of antimicrobial peptides by a variety of immune and non-immune cells. IL-17 induces nuclear factor kappa-B ligand (RANKL) and expression of intercellular adhesion molecule-1 (ICAM1), granulocyte macrophage colony-stimulating factor (GMCSF), and prostaglandin E2. Moreover, immune responses mediated by Th17 cells are highly dependent on the proinflammatory cytokine IL-23, which is produced by dendritic cells and is often referred to as the IL-23/Th17 axis [9].

Some studies found higher levels of proinflammatory cytokines in the peri-implant crevicular fluid (PICF) from patients with peri-implantitis than in crevicular fluid obtained from MP sites, while other studies were not able to find a significant difference [10].

The present study aimed to evaluate whether non-surgical treatment affects clinical parameters and local patterns of the osteo-immunoinflammatory mediators IL-17, TNF-α, and MMP-8 in PICF and biofilms during the progression of peri-implant mucositis.

Material and Methods

Study Groups

Thirty patients with peri-implant mucositis and 30 healthy people were selected for the analysis of IL-17, TNF-α cytokine, and MMP-8 production in PICF and for analysis of colonization dynamics of periodontopathogenic bacteria. All patients were divided into 3 groups: patients with healthy implants (HP), patients with untreated peri-implant mucositis affecting 2 implants (MP), and patients treated with mechanical anti-infective therapies for mucositis (TP), which involved mechanical debridement using abrasive sodium carbonate air-power and resin curets, and rinsing with chlorhexidine solution 0.12% for 2 weeks.

Sampling

Sixty non-smoking subjects (30 men and 30 women aged 55-70 years) were included in this study. They received dental care at the Department of Odontology, Medical Academy of the Lithuanian University of Health Sciences. All experiments were in accordance with all the regulations approved by the Regional Bioethics Committee City of Kaunas (No. BE-2-76) and in accordance with the Declaration of Helsinki. All volunteers involved in this study signed the informed consent form approved by the Regional Bioethics Committee City of Kaunas.

All subjects presented with at least 1 completely edentulous dental arch restored with implant-supported complete dentures. Each subject had received 2 smooth titanium implants. Conical mini-abutments measuring 3 or 4 mm high had been placed and submitted to immediate load. All implants were functioning in the patient's oral cavity for at least half a year. The mean time in function was 26.3±3.9 months. All subjects were knowledgeable about their current clinical situation and the risks and benefits of the study and signed the letter of acceptance.

Clinical Examination

Patients with diagnosis of peri-implant mucositis had implants with probing depths of 0-3 mm, including marginal bleeding,

without suppuration or radiographic signs of bone loss. Patients with healthy implants had implants with probing depths of 0-3 mm, no marginal bleeding or suppuration was present, and no radiographic bone loss was observed [11].

We excluded patients with previous conservative or surgical peri-implant tissue therapy, relevant systemic diseases, or use within the 6-month period prior to the study of any type of antibiotics, anti-allergy medications, anti-inflammatory drugs (NSAID), antioxidants, or oral antiseptics. Smokers and pregnant or lactating patients were also excluded from the study population.

Probing depth (PD) parameters were measured at 6 different locations around the implant (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual, and disto-lingual). For the following measurements, a periodontal probe was used (PCP 15, Stoma Storz am Mark, Germany). Marginal bleeding index (MBI) was assessed as the presence (1) or absence (0) of bleeding upon tissue contact with the periodontal probe. The plaque index (PI) was determined as the presence (1) or absence (0) of plaque. The suppuration index determined whether spontaneous suppuration or suppuration on probing occurred. The distance between the mucosal margin and the bottom of the sulcus were assessed by probing depth. A radiographic positioner was used to take intraoral periapical radiographs. Paralleling technique was used for these measurements. Clinical examinations were performed by the same examiner (V.O.S.), who was previously trained and calibrated [10].

Peri-Implant Crevicular Fluid Collection

In subjects from all groups, 2 or 3 sites around the implant were chosen for PICF collection. Those sites were isolated (cotton rolls were used for this procedure) and gentle drying was applied to the surfaces. A capillary tube was used for PICF collection from the peri-implant sulcus. The duration of the procedure was 30 s to avoid traumatizing the tissues [12]. Samples with blood were marked as contaminated and were eventually discarded. Microfuge tubes containing 200 µl phosphate-buffered saline and 10 µl of protease inhibitor (Sigma-Aldrich, St. Louis, MO, USA) were used for PICF samples placement for 45 min. Samples were centrifuged at 3000 g for 5 min. Supernatant was frozen at -70°C until further analysis.

Quantification of Cytokines

PICF samples were centrifuged at 5000 g for 15 min at 4°C. Enzymatic immunosorbent assay (ELISA) was used for the analysis of each PICF sample to determine IL-17 levels, according to the manufacturer's recommendations (Quantikine, R D Systems, Minneapolis, MN, USA). Briefly, 100 µl of detection antibody was added to all wells, except the blank wells, mixed

gently, and incubated overnight (16-24 h) at room temperature. Plates were washed 3 times. Standards and samples were added to wells in duplicate. The plates were washed again after incubation and then re-incubated with 100 µl of conjugate for 60 min at room temperature. Plates were washed again for 3 times, and 100 µl substrate was added and re-incubated for 15 min at room temperature in the dark. We used 50 µl stop solution to stop the reaction. Then, the color was measured in an automated microplate spectrophotometer (Microplate Reader/Model 3550, Bio Rad). The measuring units of cytokines were picograms/microliter (pg/µl). Results were calculated using standard curves created in each assay. ELISA assays were carried out in a blind fashion.

The levels of TNF-α in PICF samples were determined by enzyme amplified sensitivity immunoassay (EASIA) method. Human TNF-α EASIA kits (BioSource Europe S.A., Belgium) were used for the in vitro quantitative determination of TNF-α concentrations in the PICF. A calibration curve was plotted and TNF-α concentration (U/l) in samples was determined by interpolation from the calibration curve. The experiments with samples for each patient were performed as quadruplicates.

Protein Production

MMP-8 concentrations in 10× diluted samples of PICF were measured by ELISA (Biotrak MMP-8 human ELISA system; Amersham Pharmacia Biotech, Buckinghamshire, UK; Quantikine human TIMP-1 immunoassay; RD Systems, Minneapolis, MN, USA) according to the manufacturer's protocols. The average lower limits of detection were 0.032 and 0.08 ng/ml, respectively. All ELISAs were performed in duplicate.

Microbial Sampling

For collection of the subgingival biofilm, endodontic paper points were inserted into the periodontal sulcus or pocket until resistance from the base of the pocket was felt and were kept in place for 30 s. After sampling, sterile tubes were used for storage of the points and paper strips. The tubes contained 300 µl of RNAlater (RNAlater; Sigma-Aldrich, St. Louis, MO) and were stored shortly after collection at -80°C until processed. We used the molecular genetic assay for combined identification of additional periodontopathogenic bacterial species as an extension of the micro-IDent test. The micro-IDent plus (Germany) assay was performed. It is based on the DNA-STRIP technology and allowed as an extension test of the micro-IDent by HAIN LIFESCIENCE, the combined molecular genetic identification of periodontopathogenic bacterial species, namely *Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans), Porphyromonas gingivalis (P. gingivalis), Treponema denticola (T. denticola), Fusobacterium nucleatum (F. nucleatum), Prevotella intermedia (P. intermedia), Tannerella forsythia*

Table 1. Clinical data of patients with healthy implants (HP) and peri-implant mucositis patients (MP).

Mean (SD)	HP, n=30		MP, n=30		P value
Age, years	63.1	(1.7)	63.0	(2.0)	>0.05
Probing depth, mm	1.4	(0.2)	3.0	(0.2)	<0.001
Plaque index	0		0.53	(0.31)	<0.001
Marginal bleeding index	0		0.26	(0.07)	<0.001

SD – standard deviation; *P* value by *t* test.

Table 2. Significance of differences in probing depth, plaque index, and marginal bleeding index of healthy subjects, and the mucositis patients untreated and treated groups.

Study groups	Probing depth, mm	Plaque index	Marginal bleeding index
	Median [25-75%]		
HP, n=30	1.4 [1.2-1.6]*,**	0*,**	0*,**
MP, n=30	3.0 [2.8-3.2]*	0.6 [0.3-0.8]*,***	0.28 [0.27-0.29]*,***
TP, n=30	2.95 [2.8-3.1]**	0.1 [0.06-0.11]**,***	0.03 [0-0.09]**,***
	χ^2=61,071, df=2 ,P<0.001	χ^2=57.95, df=2, P<0.001	χ^2=65.045, df=2, P<0.001
	*,**,*** P<0.001		

χ^2 – by Kruskal-Wallis test, multiple comparison by Mann-Whitney test; df – degrees of freedom; HP – healthy subjects; MP – patients with untreated peri-implant mucositis; TP – patients treated with mechanical anti-infective therapies for mucositis.

(T. forsythia), Parvimonas micra (P. micra), Campylobacter rectus (C. rectus), Eubacterium nodatum (E. nodatum), Eikenella corrodens (E. corrodens), and Capnocytophaga sp. (C. gingivalis, C. ochracea, C. sputigenal) (C. sputigenal). The whole procedure was divided into 3 steps: DNR isolation from subgingival samples using a Geno type DNA isolation kit (Germany), with multiplex amplification with biotinylated primers and reverse hybridization. The detection level was determined as 10^3 bacteria per sample.

Reagents

Hank's balanced salt solution (Sigma Chemical Co. (St. Louis, Missouri, USA) and plastic vials were used from Carl Rot GmbH & Co KG (Karlsruhe, Germany).

Statistical Analysis

Statistical analysis of the data was performed using SPSS 22.0 for Windows for storage and analysis of data. All parametric data are expressed as the mean and standard deviation median (25-75%). The Kolmogorov-Smirnov test was used for determination of quantitative data distribution. The *t* test was used when the distribution of variables was normal, and there was a comparison of quantitative sizes of 2 independent samples. For non-normally distributed variables, the Mann-Whitney

U test was used. The Kruskal-Wallis test was used for comparison of non-normally distributed variables. Chi-square (χ^2) tests were used to compare frequencies of qualitative variables. Linear relationships of the variables were assessed using Spearmen's correlation coefficient. For the highest accuracy in the minimally false positive and minimally false negative results assessment, an ROC (receiver operating characteristics) curve method was applied. Logistic regression analysis was performed to determine the odds ratio predictive value. Differences between groups were considered significant at *P* less than 0.05 (*P*<0.05).

Results

The means and standard deviations of the clinical indices of the studied groups are summarized in **Table 1**. The mean age of the MP and HP groups (63.1±1.1 years and 63.0±1.0 years, respectively) did not differ significantly (*P*>0.05). The PD mean was greater in the MP group in comparison to the HP group, at 3.0 mm and 1.5 mm, respectively (*P*<0.001). The MP group had a significantly higher PI and MBI in comparison to the HP group (*P*<0.001). There was no suppuration observed in any of the groups.

When compared to MP and HP groups, significant decreases in MBI, PI, and PD were observed in the TP group (*P*<0.001)

Table 3. The levels of MMP-8, IL-17, and TNF-α in peri-implant crevicular fluid of healthy subjects and the mucositis patients untreated and treated groups.

Study groups	MMP-8	IL-17	TNF-α
	Median [25-75%]		
HP, n=30	0.99 [0.68-1.4]*,**	1.87 [0.83-3.24]*,**	0.7 [0.53-0.74]*,**
MP, n=30	3.27 [2.46-7.24]*,***	17.94 [14.44-20.56] *,***	3.08 [2.69-3.82]*,***
TP, n=30	1.70 [0.53-3.05]**,***	6.35 [4.43-6.75] **,***	1.52 [1.17-1.88]**,***
	χ^2=39.671, df=2, P<0.001	χ^2=77.615, df=2, P<0.001	χ^2=79.137, df=2, P<0.001
		*,**,*** P<0.001	

χ^2 – by Kruskal-Wallis test, multiple comparisons by Mann-Whitney test; df – degrees of freedom. HP – healthy subjects; MP – patients with untreated peri-implant mucositis; TP – patients treated with mechanical anti-infective therapies for mucositis.
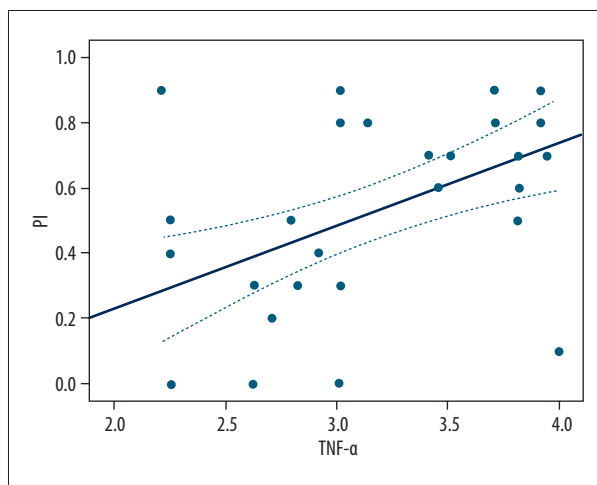
Figure 1. Spearman correlations for clinical parameter plaque index and cytokine TNF-α levels in the samples of peri-implant crevicular fluid of the mucositis patients untreated group. r=0.479, P=0.007; y=-0.273 + 0.252*x. PI – plaque index.
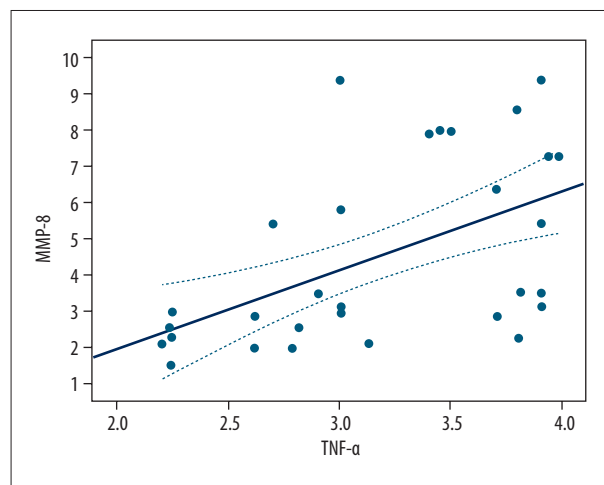
Figure 2. Spearman correlations for cytokine TNF-α levels and MMP-8 in the samples of peri-implant crevicular fluid of the mucositis patients untreated group. r=0.587, P=0.001; y=-2.427+2.178*x. χ^2 – chi-square test; df – degrees of freedom; HP – healthy subjects; MP – patients with untreated peri-implant mucositis; TP – patients treated with mechanical anti-infective therapies for mucositis.

(**Table 2**). The HP group had lower levels of IL-17, TNF-α, and MMP-8 in the PICF in comparison to the MP and TP groups, with a statistically significant difference (P<0.001) (**Table 3**).

Figures 1 and 2 show the results of correlation analysis for clinical parameters and cytokine levels in PICF. TNF-α levels in the samples of PICF showed a moderate correlation with clinical parameters such as PI (P=0.007) and MMP-8 levels (P=0.001) in the MP group. The levels of IL-17 and MMP-8 in PICF did not correlate with any clinical parameters. Data for the identification of bacteria when a bacterial species was found in at least 1 of the 3 assayed sites.

Comparisons between groups are shown in **Table 4**. Logistic regression was used to predict bacterial colonization according to cytokine activity in PICF. A significant association was found in patients with mucositis associated with *Parvimonas micra*, as TNF-α in PICF was significantly higher (P=0.034) than in patients without *Parvimonas micra* (**Figure 3, Table 5**). Patients with mucositis and *Parvimonas micra* had significantly higher levels of TNF-α in PICF (P<0.05) than patients without *Parvimonas micra* with corresponding median values [25-75%] 2.75 [2.34-3.31] and 3.61 [2.99-3.84], P=0.034.

Based on the ROC curve, we can predict TNF-α in PICF optimal cut-off value of 2.8, with odds ratio of 11.2. If *Parvimonas micra* has been detected, then TNF-α would be >2.8.

Table 4. Differences in bacteria *A. actinomycetemcomitans (A. a.), P. gingivalis (P. g.), T. denticola (T. d.), F. nucleatum (F.), P. intermedia (P. i.), T. forsythia (T. f.), P. micra (P.m.), C. rectus (C. r.), E. nodatum (E. n.), E. corrodens (E. c.), C. sputigenal (C. s.)* frequencies in the study group patients.

Bacteria	Study groups			χ^2, df=2, P
	HP, n=30	MP, n=30	TP, n=30	
	%			
Aggregatibacter actinomycetemcomitans (A.a.)	0*,**	30.0*	26.7**	10.588; 0.005 *,**P<0.05
Porphyromonas gingivalis (P.g.)	0*,**	46.7*	43.3**	19.365; <0.001 *,**P<0.05
Prevotella intermedia (P.i.)	3.3	13.3	10.0	1.921; 0.383
Tannerella forsythia (T.f.)	0*,**	20.0*	16.7**	6.421; 0.04 *,**P<0.05
Treponema denticola (T.d.)	0*,**	43.3*	36.7**	16.705; <0.001 *,**P<0.05
Parvimonas micra (P.m.)	0*,**	60.0*	56.7**	28.706; <0.001 *,**P<0.05
Fusobacterium nucleatum/periodonticum (F.n.)	3.3*,**	100.0*,***	50.0**,***	56.117; <0.001 *,**,***P<0.05
Campylobacter rectus (C.r.)	6.7	26.7	13.3	4.737; 0.094
Eubacterium nodatum (E.n.)	10.0	20.0	16.7	1.184; 0.553
Eikenella corrodens (E.c.)	16.7*,**	53.3*	46.7**	9.631; 0.008 *,**P<0.05
Capnocytophaga spp. (C.s.)	3.3*,**	80.0*	66.7**	40.267; <0.001 *,**P<0.05

χ^2 – Chi square test; df – degrees of freedom; HP – healthy subjects; MP – patients with untreated peri-implant mucositis; TP – patients treated with mechanical anti-infective therapies for mucositis.
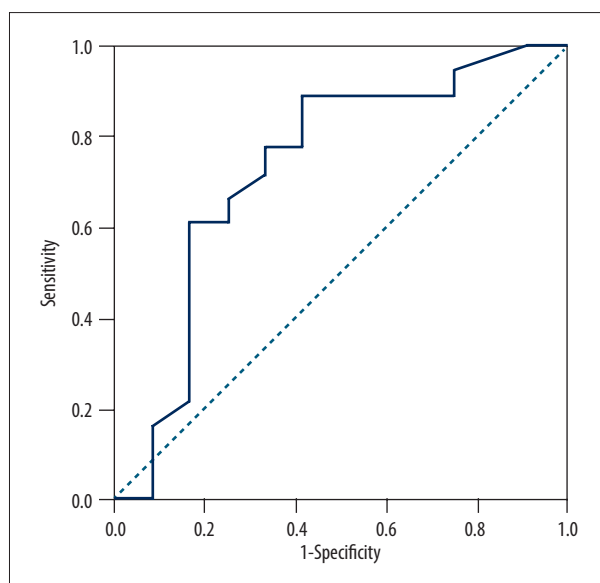
Figure 3. ROC curve of prognosis bacteria *Parvimonas micra* by using logistic regression method according to optimal cut-off value of TNF-α in peri-implant crevicular fluid of the mucositis patients untreated group.

Discussion

It is acknowledged that MP is a predecessor of peri-implantitis. Therefore, to prevent MP progression to peri-implantitis, the establishment of an early diagnosis and treatment must occur [3]. During inflammation, the affected region has an increased blood flow and vascular permeability, with associated fluid exudation, including leukocyte migration and accumulation from blood vessels into the tissues. During this inflammatory process, osteoclasts are activated, which can lead to peri-implant pockets formation, which can encourage growth of bacteria. This results in increased probing depths and bone loss [10,11].

Proinflammatory cytokines (TNF-α, IL-1β) have important roles in natural immunity and inflammation and are important mediators of inflammatory host reactions to infection and other inflammatory stimuli. Immune reactivity guidelines can be used for peri-implant tissues stage evaluation.

In bone resorption stimulation, TNF-α and IL-1β act synergistically. They also can enable the migration of inflammatory cells

Table 5. Prognosis bacteria *Parvimonas micra* by using logistic regression method according to TNF-α in peri-implant crevicular fluid of the mucositis patients untreated group.

Parvimonas micra	TNF-α, median [25-75%], mean rank	Area under the ROC curve, %	Optimal cut-off value of TNF-α	Sensitivity/specificity, %	Parvimonas micra, n (%)	OR [95% CI]
No	2.75 [2.34-3.31], 11.33				5 (41.7)	1.0
Yes	3.61 [2.99-3.84], 18.26	73.1	<2.8	88.9/58.3	16 (88.9)	11.2 [1.735-72.3]
P value	0.034*				0.006**	

ROC – receiver pperating characteristics; OR – odds ratio; CI – confidence interval; * *P* value by Mann-Whitney test; ** asymptotic P value.

into tissue and participate in the acute-phase response against infection and pathogenesis of peri-implant destruction [13]. These cytokines stimulate the inflammation and tissue destruction processes and increasing bone resorption [13]. They also stimulate the release of MMP, which causes degradation of proteins of the extracellular matrix and affect the local and systemic manifestation of inflammation [13].

Th1, Th2, and Th17 play an important role in the pathogenesis of peri-implantitis, although the details are unclear. Activation of Th1 subpopulation cells by IL12 results in production of IL-2 and IFN-γ. These cytokines predominantly stimulate the cell immune response mediated by cytotoxic T cells, macrophages, and natural killer (NK) cells [14]. On the other hand, activation of Th2 subpopulation cells is followed by the production of IL-4, IL-5, IL-6, and IL-10, which stimulates a predominantly humoral immune response and inhibits the cells immune response [14]. Th17 is a newly discovered subtype of CD+4 T cells that participate in immunity to bacteria and in the pathogenesis of autoimmune processes [14]. The level of IL-17, the most important representative of this type of cytokine, is increased during gingivitis and periodontal diseases [14]. The levels of IL-17 and IL-10 are increased in patients with peri-implant compared to individuals with healthy peri-implant tissues, and the results showed that the highest concentrations of IL-17 and IL-10 were observed in patients with peri-implant mucositis and peri-implantitis, respectively [15].

In our study in PICF, the levels of TNF-α and IL-17 were significantly lower in TP compared to MP (*P* <0.001). Although the therapy of peri-implant mucositis is considered necessary to avoid peri-implantitis, all clinicians should focus on the prevention of mucositis, taking into account the findings of Ribeiro et al that during 21-day period of plaque accumulation, gingiva around teeth develop weaker inflammatory response in comparison to soft tissues surrounding implants [16].

Our study revealed moderate correlations between biochemical parameters in the samples of PICF and between the cytokine

levels of TNF-α and clinical parameters (PI) (*P*=0.007) and MMP-8 levels (*P*=0.001) in the group of patients with MP.

The aforementioned results agree with a study that found a correlation between IL-6 and TNF-α in groups with peri-implant mucositis, but no signs of interproximal alveolar bone loss, in comparison to individuals with peri-implantitis. This suggests that the presence of proinflammatory cytokines is correlated with peri-implant disease [17].

A major role is played by the matrix metalloproteinase (MMPs) in various tissue-destructive inflammatory processes in which most of the basement membrane and peri-cellular components are degraded. Therefore, MMP-8 can be used as an adjunctive diagnostic biomarker in peri-implant diagnostics [18]. Similar possibilities might be offered by PICF MMP-8, as its levels were positively associations with probing depths and gingival index in loaded implants [18]. Early implant failures can occur due to MMP-8 gene polymorphisms in its promoter region. MMP-8 (Collagenase-2), which is an important mediator of LPS induced inflammation, and also other proinflammatory transmitters, are activated by the MMPs [19].

MMP-8 levels are increased and converted to active form in peri-implantitis-affected PICF and accordingly differ from MMP-8 detected in PICF from peri-implant mucositis-affected and healthy oral implants [18]. In particular, a stronger inflammatory response to accumulation of plaque is developed by peri-implant soft tissues in contrast to their gingival counterparts when evaluated by potential biomarkers, including MMP-8 [18]. Formation of biofilm and development of gingivitis and peri-implant mucositis are highly associated, and this was found to be reversible by addressing gingival crevicular fluid (GCF) and PICF biomarker MMP-8. A prospective study revealed that MMP-8 was an early sign of peri-implant inflammation, and assessment of PICF MMP-8 was advantageous in controlling the course of peri-implant disease [18].

A recent cross-sectional study was done, in which scientists analyzed 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*), showing that MMP-8 levels were not significantly different among the investigated groups [20]. Healthy and peri-implantitis-affected implants were not compared based on the status of natural teeth and no treatment was given, which makes it difficult to compare the results with other studies in which peri-implantitis was treated with surgery and PICF levels of bone markers were analyzed, and in which a significant reduction of MMP-8 and IL-6 and bone resorption markers were detected [21,22].

MMP-8 is the first line of innate immune response, and, according to our findings, MMP-8 is a viable potential biomarker that should be used along with clinical parameters for the tracking of peri-implant health and disease. A viable scheme would be to analyze PICF MMP-8 together with bone loss biomarkers such as soluble RANK ligand, osteoprotegerin (OPG), and sclerostin, which have been found to be significantly increased in patients with peri-implantitis in comparison to the patients with healthy peri-implant tissues, as prognostic biomarkers in peri-implantitis [23,24].

Conclusions

Evaluation of inflammatory cytokines levels in peri-implant diseases can permit the identification of active disease, which may be a tool for early diagnosis, prevention, and treatment. MMP-8 was found to be an early sign of peri-implant inflammation. Levels of TNF-α and IL-17 were significantly lower in the treated group patients compared to peri-implant mucositis before treatment.

Department and Institution Where Work Was Done

Department of Dental and Maxillofacial Orthopedics, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania.

Declaration of Figures Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

References:

1. Sfasciotti GL, Trapani CT, Powers RM. Mandibular ridge augmentation using a mineralized ilium block: A case letter. J Oral Implantol. 2016;42:215-19

2. Academy of Osseointegration. 2010 Guidelines of the Academy Osseointegration for the provision of dental implants and associated patient care. Int J Oral Maxillofac Implants. 2010;25:620-27

3. Ata-Ali J, Flichy-Fernández AJ, Alegre-Domingo T, et al. Clinical, microbiological, and immunological aspects of healthy versus peri-implantitis tissue in full arch reconstruction patients: A prospective cross-sectional study. BMC Oral Health. 2015;15:43

4. Barbato L, Francioni E, Bianchi M, Mascitelli E, et al. Periodontitis and bone metabolism. Clin Cases Miner Bone Metab. 2015;12:174-77

5. Janska E, Mohr B, Wahl G. Correlation between peri-implant sulcular fluid rate and expression of collagenase2 (MMP8). Clin Oral Investig. 2016;20:261-66

6. Ata-Ali J, Candel-Marti ME, Flichy-Fernández AJ, et al. Peri-implantitis: Associated microbiota and treatment. Med Oral Patol Oral Cir Bucal. 2011;16:e937-43

7. Nowzari H, Phamduong S, Botero JE, et al. The profile of inflammatory cytokines in gingival crevicular fluid around healthy osseointegrated implants. Clin Implant Dent Relat Res. 2012;14:546-52

8. Kim KW, Kim HR, Kim BM, et al. Th17 cytokines regulate osteoclastogenesis in rheumatoid arthritis. Am J Pathol. 2015;185:3011-24

9. Pradeep AR, Hadge P, Chowdhry S, et al. Exploring the role of Th1 cytokines: Interleukin-17 and interleukin-18 in periodontal health and disease. J Oral Sci. 2009;51:261-66

10. Severino VO, Beghini M, de Araújo MF, et al. Expression of IL-6, IL-10, IL-17 and IL-33 in the peri-implant crevicular fluid of patients with peri-implant mucositis and peri-implantitis. Arch Oral Biol. 2016;72:194-99

11. Lang NP, Berglundh T; Working Group 4 of Seventh European Workshop on Periodontology. Periimplant diseases: where are we now? – Consensus of the Seventh European Workshop on Periodontology. J Clin Periodontol. 2011;38(Suppl. 11):178-81

12. Abbas AK, Janeway CA Jr. Immunology: Improving on nature in the twenty-first century. Cell. 2000;100:129-38

13. Fonseca FJ, Moraes Junior M, Lourenço EJ, et al. Cytokines expression in saliva and peri-implant crevicular fluid of patients with peri-implant disease. Clin Oral Implants Res. 2014;25:e68-72

14. Bunte K, Beikler T. Th17 cells and the IL-23/IL-17 axis in the pathogenesis of periodontitis and immune-mediated inflammatory diseases. Int J Mol Sci. 2019;20:3394

15. Farhad SZ, Rezazadeh F, Mohammadi M. Interleukin -17 and interleukin-10 as inflammatory and prevention biomarkers in periimplant diseases. Int J Prev Med. 2019;10:137

16. Ribeiro FV, Casati MZ, Casarin RC, et al. Impact of a triclosan-containing toothpaste during the progression of experimental peri-implant mucositis: Clinical parameters and local pattern of osteo-immunoinflammatory mediators in peri-implant fluid. J Periodontol. 2018; 89(2):203-12

17. Teixeira MKS, Lira-Junior R, Telles DM, et al. Th17-related cytokines in mucositis: Is there any difference between peri-implantitis and periodontitis patients? Clin Oral Implants Res. 2017;28:816-22

18. Basegmez C, Yalcin S, Yalcin F, et al. Evaluation of periimplant crevicular fluid prostaglandin E2 and matrix metalloproteinase-8 levels from health to peri-implant disease status: A prospective study. Implant Dent. 2012;21:306-10

19. Costa-Junior FR, Alvim-Pereira CC, Alvim-Pereira F, et al. Influence of MMP-8 promoter polymorphism in early osseointegrated implant failure. Clin Oral Investig. 2013;17:311-16

20. Sakalauskiene J, Giedrimiene D, Gleiznys D, et al. Peripheral blood leukocytes IL-1β cytokine hyper-reactivity in chronic periodontitis. Med Sci Monit. 2016;22:4323-29

21. Wang HL, Garaicoa-Pazmino C, Collins A, et al. Protein biomarkers and microbial profiles in peri-implantitis. Clin Oral Implants Res. 2016;27:1129-36

22. Wohlfahrt JC, Aass AM, Granfeldt F, et al. Sulcus fluid bone marker levels and the outcome of surgical treatment of peri-implantitis. J Clin Periodontol. 2014;41:424-31

23. Rakic M, Struillou X, Petkovic-Curcin A, et al. Estimation of bone loss biomarkers as a diagnostic tool for peri-implantitis. J Periodontol. 2014;85:1566-74

24. Faot F, Nascimento GG, Bielemann AM, et al. Can peri-implant crevicular fluid assist in the diagnosis of peri-implantitis? A systematic review and meta-analysis. J Periodontol. 2015;86:631-45