

Comparison of the Levels of Tumor Necrosis Factor- α and Interleukin-17 in Gingival Crevicular Fluid of Patients with Peri-implantitis and a Control Group with Healthy Implants

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ABSTRACT

Peri-implantitis is a multi-factorial disease involving peri-implant tissues and resulting in therapeutic failure. Inflammatory mediators and cytokines in the Gingival Crevicular Fluid (GCF) have pivotal roles in the disease pathogenesis and could be used for disease monitoring. Therefore, the present study was conducted to compare the GCF levels of TNF- α and IL-17 between patients with peri-implantitis and healthy implants.

In this case-control study, 24 patients with peri-implantitis and 18 individuals with healthy implants referring to faculty of dentistry in Tehran University of Medical Sciences were selected. GCF was collected by paper cons number 30. Samples were preserved in PBS - 70°C. TNF- α and IL-17 levels in GCF were determined by ELISA method. Data were analyzed by SPSS software version 13, using descriptive indices and independent t tests.

Mean probing depth in peri-implantitis and control groups were 6.2 ± 1.1 and 3.7 ± 1.6 mm respectively. Mean level of IL-17 in patients with peri-implantitis was significantly more than the control group (19.8 ± 16.0 versus 9.3 ± 8.4 pg per site in 40 seconds, $p=0.016$). Also, mean level of TNF- α in patients with peri-implantitis was more than control group (39.0 ± 3.9 versus 14.5 ± 9.0 pg per site in 40 seconds, $p = 0.000$).

The significant higher levels of TNF- α and IL-17 in patients with peri-implantitis compared to control group indicated the pivotal role of these cytokines in peri-implantitis and could be suggested as diagnostic markers and in future possibly for immunomodulatory treatments.

Keywords: IL-17; Implant; Peri-implantitis; TNF- α

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INTRODUCTION

Nowadays with improvement of dental implantology science, osseointegrated implants show a considerable durability, however failures are not completely avoidable.¹ Peri-implant tissues are

susceptible to bacterial infections and consequently induce inflammation and peri-implantitis, which indicate a periodontal disease similar to chronic periodontitis.

The inflammatory process in response to bacterial infection is mediated by proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF- α), interferon-gamma (IFN- γ), and interleukins (IL)-1 β , IL-6, IL-12, and IL-17. These cytokines induce tissue destruction and bone resorption by activation of collagenase and also through the receptor activator of nuclear factor-kappa B ligand (RANKL) which stimulate osteoclast differentiation.²⁻⁴

Mucositis is an infectious disease that results in a reversible inflammatory process restricted to the soft tissues around osseointegrated implants, while the periimplantitis is an irreversible reaction that affects the soft tissues and the supporting bone around the implant in occlusal function. Perimplantitis is characterized by edema, erythema, suppuration, pain, probing depth more than 6 mm, bleeding on probing, excessive mobility, perimplant radiolucency and progressive alveolar bone resorption.⁵ It has been shown in different studies that establishment of bacteria on dental plaque induces the host immune response and results in tissue destruction.⁶⁻⁸

Cytokines are the most important mediators of inflammation, which play a pivotal role in pathogenesis of periodontal diseases, and can induce periimplantitis. These inflammatory mediators appear in patients' GCF, providing the opportunity of non-invasive sampling and evaluation of cytokines levels in GCF. By sampling from the GCF it has been facilitated to detect the periodontal diseases in early and treatable stages, covering the defects of conventional diagnostic procedures, such as radiography and probing.⁹⁻¹⁴ Using the cytokine assay in GCF in recent years periodontal researches have been concentrated on analysis of GCF cytokines, such as IL-1 β , TNF- α , and other inflammatory markers to describe the pathogenesis more efficiently and through discovering different markers capable of predicting early diagnosis of periimplantitis and anticipation of high risk patients.¹⁵⁻¹⁸ Different studies on cytokine single nucleotide gene polymorphisms and susceptibility to development of periimplantitis have shown inconsistent and controversial results.¹⁸⁻²⁵ However studies which compared the cytokines levels of GCF in patients with periimplantitis and healthy implants have indicated the

higher levels of pro-inflammatory cytokines in patients with periimplantitis compared to healthy implants. These cytokines induce stimulation and activation of osteoclasts and bone resorption.²⁶⁻³⁰ Curtis DA et al and Murata M et al in peri-implant crevicular fluid analysis have shown that inflammatory cytokines, such as IL-1 β , could be an appropriate markers in monitoring perimplant tissue during the inflammation and health.^{31,32}

TNF- α is an osteoclast activating cytokine, which induce bone resorption directly by increasing the pool size of marrow osteoclast precursors. In addition TNF- α induce RANKL mRNA, inhibit OPG mRNA and finally stimulating bone resorption.^{3,33} It has been shown that mechanical antimicrobial treatments improve clinical parameters of involved implants, through reduction of GCF level of this cytokine.^{34,35}

IL-17 is another osteoclast activating cytokine, increasing in periodontal diseases such as chronic periodontitis, with an important role in regulation of other destructive cytokines.^{36,37} A recent study has shown that GCF level of IL-17 enhances periimplantitis too.³⁸

However, there is not enough evidence that TNF- α and IL-17 are associated with development of periimplantitis. Therefore, the present study was performed to compare the GCF levels of TNF- α and IL-17 between peri-implantitis patients and people with healthy implants.

MATERIALS AND METHODS

In this case-control study, GCF levels of TNF- α and IL-17 were compared between patients with periimplantitis and people with healthy implants, referring to faculty of dentistry in Tehran University of Medical Sciences in 2010. Participants were selected by available sampling method. Inclusion criteria were totally or partially edentulous subjects treated with at least one screw-shaped, machined-surface titanium implant in function for 1 year. Subjects were non-smokers, non-pregnant and non-breast lactating women, systemically and periodontal healthy or periodontal treated, and engaged in supportive periodontal therapy (for partially edentulous individuals). Exclusion criteria were the use of antibiotics, anti-inflammatory drugs, or local antimicrobial agents within the preceding 6 months. Patients were informed about the characteristics of the

TNF- α and IL-17 in GCF of peri-implantitis patients

study and gave their written consent to the described procedures. At first, all participants signed an informed consent voluntarily and demographical variables were recorded. Peri-apical radiography with parallel technique was prepared to detect bone resorption. Williams probe was used to assess clinical parameters including probing depth, suppuration (Sup) and bleeding on probing (BOP). One week later, GCF sampling was performed through deepest pocket. If more than one site with similar depth was involved, sampling was done through mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual and distolingual sites respectively.

Biofilm was removed by sterile cotton rolls, and sampling was performed by paper cons number 30, 4 times with 3-minute intervals, each time for 10 seconds through the depth of 3 mm. Blood contaminated samples were eliminated, to be sampled again. Afterward, cons were set in tubes containing 200 μ l of phosphate buffer and preserved in -70°C, until the evaluation of levels of TNF- α and IL-17.

The quantitative sandwich enzyme technique of ELISA (QuantiKine High Sensitivity kit from R&D Systems, Minneapolis, Minn) was used to evaluate the concentration of TNF- α and IL-17 in samples. Test was performed considering kit instructions and then optical

density was read by ELISA reader model Awareness-Statfax2100.

Finally, data were analyzed by SPSS software version 13. Kolmogrov-Smirnov test was used to assess the normality of distribution for TNF- α and IL-17 levels. Then, independent t test was used to compare the GCF levels of TNF- α and IL-17 between case and control groups.

RESULTS

This study was carried out on 42 participants, including 24 patients with peri-implantitis (14 women and 10 men) and 18 individuals with healthy implants (11 women and 7 men). Participants aged 28 to 57 years and mean ages in peri-implantitis and control groups were 44.4 \pm 6.6 and 40.8 \pm 8.6 years respectively. Independent t test showed that this difference was not significant ($t=1.553$, $p=0.128$).

Mean probing depth in perimplantitis and control groups were 6.5 \pm 1.1 and 3.7 \pm 1.5 mm respectively. Prevalence of suppuration and bleeding on probing in peri-implantitis group were 29.2% and 100% respectively, while there was no sign and symptoms in control group.

Table 1. Kolmogrov-Smirnov test for normality of distribution of TNF- α and IL-17 in perimplantitis and control groups.

Groups		TNF α	IL17
Perimplantitis group	Z	0.501	1.128
	P	0.963	0.157
Control group	Z	0.589	0.864
	P	0.878	0.444

Table 2. Independent t test for comparison of level of TNF- α and IL-17 between perimplantitis and control groups.

Variables	t	Df	P-value
IL-17	-2.516	40	0.016
TNF- α	-8.549	40	0.000

Table 3. Independent t test for evaluation of the relationship between the levels of TNF- α and IL-17 and gender in perimplantitis group.

Variables	t	df	P-value	Mean difference
IL-17	-0.585	40	0.562	-2.62
TNF- α	0.391	40	0.698	1.89

Regarding ELISA test results, mean levels of IL-17 in perimplantitis and control groups were 19.7 ± 16.0 and 14.5 ± 8.9 pg/site in 40 seconds of sampling respectively. Also, mean level of TNF- α was 38.9 ± 9.3 and 14.5 ± 8.9 pg/per site in 40 seconds of sampling respectively. Kolmogorov-Smirnov test showed that levels of IL-17 and TNF- α were normally distributed (Table 1). Independent t test showed that GCF levels of both TNF α ($t=-8.549$, $p=0.000$) and IL-17 ($t=-2.516$, $p=0.016$) in perimplantitis group were significantly higher than the control group (Table 2).

Pearson correlation test showed that GCF levels of IL-17 and TNF- α were not correlated with each other ($r=0.154$, $P=0.331$). Also, GCF level of TNF- α and IL-17 were not related to patient's age ($r=0.209$, $P=0.185$ for TNF α and $r=-0.041$, $P=0.795$ for IL-17) and gender ($t=0.391$, $P=0.698$ for TNF α and $t=-0.585$, $p=0.562$ for IL-17) (Table 3).

GCF level of IL-17 in perimplantitis group was related to probing depth ($r=0.521$, $P=0.009$), but the relationship between the level of TNF- α and probing depth was not significant ($r=-0.334$, $p=0.111$).

DISCUSSION

The present study evaluated 24 patients with perimplantitis and 18 individuals with healthy implants and the results showed that mean GCF levels of TNF- α and IL-17 in perimplantitis group were significantly higher in comparison to control group. GCF levels of TNF- α and IL-17 was not correlated with each other and also the patient's age and gender were not correlated in both groups. Probing depth in perimplantitis group was significantly associated with the elevated level of IL-17, but not the level of TNF- α .

Although the role of immune system in chronic periodontitis has been demonstrated, it has not been described very well yet for perimplantitis. Page and colleagues stated that progression of periodontal diseases requires a bacterial infection, local rise of pro-inflammatory cytokines, matrix metalloproteinase and prostaglandins and reduction of anti-inflammatory cytokines such as IL-10, TGF- β 1 and matrix metalloproteinase inhibitor. Therefore the local balance of these mediators determines the level of tissue destruction.³⁸ Dependent on the stage and severity of disease, production rate of various pro-inflammatory cytokines in the GCF during perimplantitis show

variation.⁵ This allows clinicians to evaluate the GCF volume and inflammatory mediators in the GCF, as a diagnostic and prognostic method for monitoring of perimplant tissues.^{31,39}

A review study by Javed F et al on 2011 depicted the role IL-1 β , IL-6, IL-8, TNF- α and matrix metalloproteinase in patients with perimplantitis compared to control group with healthy implants²⁵. These pro-inflammatory cytokines have different pathogenesis pattern in disease progression for example; IL-1 β , IL-6 and TNF- α are synergically involved in inflammatory response to bacterial antigens, while IL-8 and MIP-1 α induce leukocyte migration.⁴⁰

Different studies have shown the key role of IL-1 β in immune response in periodontal diseases. As IL-1 β increase in GCF of patient with sever clinical symptoms and loosening of implant attachments, it is suggested that IL-1 β an appropriate marker for monitoring of progression of peri-implantitis.^{1,30,31}

Some studies have shown the role of TNF- α in periodontal diseases. TNF- α induces two mechanisms, directly (with enhancement of osteoclast precursors in bone marrow) and indirectly (with impression on OPG/RANKL system). TNF- α is also involved in bone destruction and implant failure.^{23,41,42} Most of the antimicrobial agents cure the patients through reduction of TNF- α level in the GCF.^{33,34} In spite of these results, Panagakos and colleagues could not confirm these data in a pilot study on perimplantitis patients.⁸ However, the present study has shown that the GCF level of TNF- α increases in perimplantitis. These inconsistent results could be due to different techniques which have been used in various studies.

Results of Bostanci and colleagues are inconsistent with our study and Ataoglu's study,²⁶ showed a significant correlation of TNF- α and probing depth.⁴¹

It is believed that in addition to Th₁ and Th₂ lymphocytes, Th₁₇ cells are involved in pathogenesis of periodontal diseases, through secretion of IL-17 in GCF. It regulates the function of other destructive cytokines in chronic periodontitis, resulting in the activation of osteoclasts and onset the progression of inflammation.^{35,36} In another study by Schenkein and colleagues they found a positive association between serum level of IL-17 and loosening of attachments of implants to alveoli bone and claimed that it may be involved in pathogenesis of perimplantitis too.⁴³

Severino and colleagues similar to our study results ascertained that patients with perimplantitis experience a significant increase of IL-17 in GCF in comparison with control group with healthy implants, which induces the production of other destructive cytokines.³⁷ Supporting the role of IL-17 in pathogenesis of perimplantitis, the present study showed that IL-17 increased in patients' GCF compared to control group and also the level of IL-17 correlated to probing depth.

Higher levels of TNF- α and IL-17 in GCF of patients with perimplantitis compared to control group with healthy implants, indicated the role of these cytokines in the pathogenesis of perimplant tissue destruction. It seems that both cytokines are efficient for perimplant tissue health monitoring.

The results of this study suggested the potential role for local blockers of these cytokines as preventive and therapeutic targets in the future

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