Effects of Non-Surgical Periodontal Therapy on Gingival Crevicular Fluid Levels of Interleukin-17 and Interleukin-23 in Patients with Periodontitis: A Clinical Trial

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Abstract

Background and Aim: Considering the critical role of inflammatory responses in periodontal disease, knowledge about the immune system and cytokines seems necessary. The aim of this study was to evaluate the level of IL-23 and IL-17 in gingival crevicular fluid (GCF) of patients with chronic and aggressive periodontitis, before and after non-surgical periodontal therapy.

Materials and Methods: In the present clinical trial, GCF samples were collected from 54 patients (with aggressive and chronic periodontitis) before and after the initial phase of periodontal therapy. The cytokine concentration was measured using ELISA. The Wilcoxon signed-rank test was performed to analyze the effect of non-surgical periodontal therapy on each group. The correlation between variables was investigated by the Spearman's correlation coefficient.

Results: Non-surgical periodontal therapy in both groups significantly decreased the IL-17 and IL-23 levels (P<0.05). A significant correlation was noted between the concentration of IL-23 and IL-17 before the treatment in the chronic periodontal group (P<0.05), while such a correlation was not seen in the aggressive periodontitis group (P>0.05).

Conclusion: Due to the reduction of IL-17 and IL-23 after nonsurgical periodontal therapy, these factors may play a role as mediators of periodontitis pathogenesis. Direct statistical correlation between the concentration of IL-17 and IL-23 before treatment in the chronic group shows the possible role of IL-23 in inducing Th-17 cells and production of IL-17.

Key Words: Aggressive Periodontitis, Chronic Periodontitis, Cytokines, Periodontal Debridement

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Introduction

Periodontitis is in fact the inflammatory disease of the tooth supportive structures as well as one of the main reasons for tooth loss in adults [1]. The prevalence and progression rate of periodontal disease depend on the complex interactions between the bacteria and the host immune cells [2]. The cytokines produced by the immune cells in reaction to the periodontal bacteria modulate the immune responses and have a protective or destructive role in disease progression [3].

A new class of T cells was recently recognized which are called T helper 17 (Th17) on account

of producing interleukin-17 (IL-17), and are differentiated in presence of IL-23 [3,4]. These highly pathogenic T helper cells are commonly known to produce IL-17, IL-6, and tumor necrosis factor alpha (TNF-α) cytokines, but not interferon gamma (IFN-γ) and IL-4 [5]. Multiple types of innate immune cells, in addition to Th17 cells, release IL-17, among which are gamma delta T cells, natural killer cells, and neutrophils [6,7]. In infections, IL-17 is released to eliminate extracellular bacteria and fungi, by stimulating the production of several pro-inflammatory mediators, such as IL-1, IL-6, TNF-α, nitric oxide synthase, metalloproteases, and antimicrobial peptides by fibroblasts, endothelial cells, macrophages, and epithelial cells [6].

A strict regulation of these inflammatory reactions is essential for maintaining immunological homeostasis. Lack of IL-17 affects normal bacterial dissemination, associated with reduced inflammatory mediators and neutrophil recruitment [7], while excessive form of IL-17-dependent immune responses may lead to bystander cell death and its harmful consequences [8].

IL-23 is also a cytokine belonging to the IL-12 family, which is produced by the dendritic cells and macrophages within few hours after contact with lipopolysaccharides and other microbial products [9]. It is generally recognized to provoke inflammation through IL-17-dependent or IL-17-independent pathways by inducing IL-1 and IL-6 production via activation of myeloid cells [6]. A previous study showed that elevated levels of IL-17 induced by IL-23 could result in unfavorable events during tissue injury responses in presence of additional inflammatory cytokines such as IL-1, IL-6, GM-CSF, and TNF [5].

Accordingly, Th17 and IL-23 play important roles in the pathogenesis of tissue damage by immune cells. It has been reported that IL-17 plays an important role in the etiology of bone inflammatory diseases such as periodontitis and rheumatoid arthritis which is known as a disease with a similar pattern to periodontal disease [9].

IL-17 can also be effective in development of periodontal disease via osteoclastogenesis [10]. It has been reported that IL-17 blockage after the onset of disease can effectively prevent bone and cartilage loss and decrease the clinical symptoms [11]. Interestingly, IL-23 is also necessary for the formation of osteoclasts and preservation of bone mass, although IL23 alone is insufficient to induce osteoclastogenesis [12]. However, some studies have shown a positive relationship between the amount of IL-17 with the severity of chronic periodontitis, reporting an increased level of IL-17 in the gingival crevicular fluid (GCF) collected from the areas involved with periodontitis [9,13], while some others introduced IL-17 as a cytokine with protective effects against tissue damage [14-17]. Considering the existing controversy, the role of IL-17 and IL-23 as protective or destructive factors is not clearly understood.

On the other hand, in spite of clinical differences between chronic and aggressive periodontitis, it is not obvious if these two types of periodontal diseases are completely different in serum inflammatory mediators. Some interventional studies hypothesized that treatment of periodontal disease can reduce the serum level of pro-inflammatory mediators, but the results about the effect of periodontal treatment on the serum level of pro-inflammatory biomarkers have been paradoxical [14,18,19]. Thus, this study aimed to assess the effect of non-surgical periodontal therapy on the concentration of IL-17 and IL-23 in patients with periodontal disease referred to the Periodontology Department of the Faculty of Dentistry, Shahed University, in 2013-2014.

Materials and Methods

In this clinical trial, the study participants were selected from individuals with periodontitis referred to the Periodontology Department of Shahed University from October 2013 to December 2014.

After periodontal examination, 54 systemically healthy individuals met the inclusion criteria and were recruited. The participants were classified into two groups of chronic

periodontitis (n=25) and aggressive periodontitis (n=29). The sample size was determined based on the statistical information of a similar study [20]. In the current study, type I error and power of study were considered 0.05 and 80%, respectively. The eligible patients were selected by convenience sampling.

Aggressive periodontitis was diagnosed with presence of at least three permanent teeth other than first molars/incisors with probing depth and clinical attachment loss > 5 mm in patients < 35 years of age with familial aggregation. Chronic periodontitis was diagnosed with presence of at least five teeth with a probing depth ≥ 5 mm, clinical attachment loss ≥ 3 mm, and presence of bleeding on probing [21].

The exclusion criteria were history of periodontal treatment within the previous 6 months, use of any antibiotics or immunosuppressive drugs in the past 3 months, pregnancy, smoking, and consumption of any type of medication affecting the periodontium (including medications that cause gingival hypertrophy such as calcium channel blockers). Sampling was not performed around the third molars, teeth with severe occlusal interferences, and teeth with perio-endo lesions or abscess.

Detailed explanation was given to the patients, and all the participants signed informed consent forms. The methodology of the present study was approved by the ethics committee of Shahed University, Tehran, Iran (approval code: IR.Shahed.REC.1394.297). This clinical trial was registered in the Iranian Registry of Clinical Trials (registration code: IRCT20180729040623N1).

Data collection and GCF sampling:
Periodontal examination was performed by a single examiner. The probing pocket depth and clinical attachment level were recorded in four areas around each tooth (mesiobuccal, mesiolingual, distobuccal, distolingual) by a Williams probe, and the mean value was reported for each patient. A series of full-mouth periapical radiographs were taken from each patient to diagnose periodontitis and to differentiate chronic periodontitis from aggressive periodontitis. After recording the

periodontal parameters and baseline GCF sampling to measure the concentration of cytokines before non-surgical periodontal treatment, the first phase of periodontal therapy including oral hygiene instruction, and supra- and sub-gingival scaling and root planing were performed in at least two visits for all participants. Six weeks later, all patients were re-examined, and GCF sampling was repeated.

The GCF samples were collected from two non-adjacent deepest pockets in each patient. The selected area was washed by saline, isolated by cotton rolls, and gradually dried with air spray.

After supra-gingival plaque removal with a periodontal curette, a paper strip (Periopaper, Oraflow Inc., USA) was placed inside the periodontal pocket for 30 s. Contaminated papers were discarded, and the sampling process was repeated again. Afterwards, the collected samples were transferred to an air-tight microtube and stored at -20°C.

Following the sampling process, the paper strips were stored in 150 μL of phosphate-buffered saline and centrifuged at a speed of 10,000 rpm for 30 min to extract GCF. The IL-23 and IL-17 levels were measured using a human IL-23 ELISA kit [Bender Med Systems GmbH, Austria (catalogue no. BMS2017)] and a human IL-17A ELISA kit [Bender Med Systems GmbH, Austria (catalogue no. BMS2023)] respectively, according to the instructions described in our previous studies [20, 21].

Statistical analysis:
Normal distribution of values was rejected by the Shapiro-Wilk test. For assessing the equality of variances in different study groups, the Levene's test was applied. The significance of differences within and between groups was evaluated by the Wilcoxon signed-rank test and Mann-Whitney U test. To assess any correlation between cytokine concentration and clinical parameters, the Spearman's correlation coefficient was applied. P-values less than 0.05 were considered statistically significant. All statistical analyses were carried out using SPSS version 21.0.

Results

Demographic and periodontal characteristics of the study subjects are shown in Table 1. The baseline values of probing depth (P=0.87) and clinical attachment loss (P=0.21) did not show any significant difference between the chronic periodontitis and aggressive periodontitis groups.

After non-surgical periodontal therapy, the concentration of IL-17 and IL-23 in the chronic periodontitis group significantly decreased from 1.40±1.37 to 0.96 ± 0.44 pg/mL (P< 0.05) and from 80.60 ±132 to 1.40 ± 3.63 pg/mL, respectively (P< 0.01; Table 2). The level of IL-23 and IL-17 in the GCF of aggressive periodontitis patients significantly decreased 6 weeks after phase I periodontal treatment. The level of IL-17 diminished significantly from 0.91± 0.74 to 0.51 ± 0.54 pg/mL (P<0.05) and the level of IL-23 significantly decreased from 32.27± 63.59 to 2.34 ± 4.70 pg/mL (P<0.001).

No statistical differences in IL-17 and IL-23 concentration were noted between the chronic periodontitis and aggressive periodontitis groups before the treatment (P=0.156), but after the phase I periodontal therapy, the level of IL-17 was significantly higher in the chronic periodontitis group.

However, the Spearman's rank correlation coefficient revealed that before treatment, there was a significant statistical correlation between IL-17 and IL-23 levels in the chronic periodontitis group only, and such a significant correlation was not seen in the aggressive periodontitis group (Table 3). Moreover, after treatment, no significant statistical correlation was observed between the concentration of IL-23 and IL-17 in any of the two groups. Additionally, in the aggressive periodontitis group, there was an inverse statistical correlation between clinical attachment loss and the concentration of IL-17 before the treatment.

Discussion

The current findings proved that IL-23, an IL-12 cytokine family member, potently directs the naive CD4+ T cells' development towards IL-17–producing T helper cells (Th-17); while, excessive IL-17 secretion is mainly observed in a plethora of inflammatory diseases [5]. In our previous study [21] we also demonstrated a significant difference in the IL-17 and IL-23 levels between periodontitis patients and healthy controls that raised the question to dig deeper into the role of these cytokines in periodontitis pathogenesis. Finally, according to a variety of evidence suggesting the involvement of these two cytokines in the immune-mediated tissue damage [9], in this study we investigated the alterations of IL-17/IL-23 levels in patients with periodontitis following non-surgical periodontal therapy. This study showed that after phase I periodontal therapy, the concentration of IL-17 and IL-23 in both groups of chronic and aggressive periodontitis significantly decreased. However, the level of IL-23 and IL-17 in the two groups of chronic and aggressive periodontitis was not significantly correlated neither before nor after treatment. After periodontal treatment, comparison of IL-17 and IL-23 concentrations between the two groups showed that only the concentration of IL-17 was significantly different between the aggressive periodontitis and chronic periodontitis groups, and was higher in the chronic periodontitis group. In other words, the aggressive periodontitis group showed a better response to treatment according to the reduction of IL-17 concentration.

Duarte et al, in 2010 reported that the IL-17 level in serum before treatment in generalized aggressive group was higher compared with the generalized chronic group, and a significant reduction was observed in the serum concentration of this cytokine 6 months after treatment [3]. In our study, the concentration of IL-17 in the chronic periodontitis group was higher before the treatment, although this difference was not statistically significant. The difference between the results of the current study and those of Duarte et al. [3] can be primarily related to the type of examined samples. In the current study, the GCF samples were examined; while, in the study by Duarte et al, [3] the serum samples were examined. In fact, GCF samples, in comparison with the serum samples, would give a better reflection of

Table1. Demographic and clinical characteristic of patients in chronic and aggressive periodontitis groups

Variables		Chronic periodontitis (n=25)	Aggressive periodontitis (n=29)	P value
Age	Mean ± SD	45.48 ± 9.5	28.8 ± 5.7	0.000
Gender	Female N (%)	11(44)	4(14)	0.07
	Male N (%)	14(56)	25(86)	0.07
Probing depth	Mean ± SD	6.36 ± 1.29	5.24 ± 2.17	0.87
Clinical attachment loss	Mean ± SD	6.41± 1.24	6.24± 3.56	0.21

SD: Standard deviation

Table 2. Comparison of GCF levels of IL-17 and IL-23 before and after treatment using Wilcoxon signed-rank test

Parameters	Chronic periodontitis				Aggressive periodontitis			
Cytokine	Before Mean ±SD	After Mean ±SD	Effect size	P- value	Before Mean ±SD	After Mean ±SD	Effect size	P-value
IL-17	1.4 ±1.36	0.96 ±0.44	0.256	0.048	0.91 ±0.74	0.51 ±0.54	0.367	0.022
IL-23	80.6 ±132.1	1.41±3.63	0.476	0.001	32.27 ±63.59	2.34 ±4.7	0.118	0.000

SD: Standard deviation

Table 3. Spearman's rank correlation between IL-17 and IL-23 levels in the groups pre- and post-treatment

Group	Variable		IL-17 Post treatment	IL-23 Post treatment
Chronic periodontitis	IL-17 Pre-treatment	Correlation coefficient P-value	0.154 0.495	-0.467* 0.028*
	IL-23 Pre-treatment	Correlation coefficient P-value	0.025 0.913	-0.152 0.499
Aggressive periodontitis	IL-17 Pre-treatment	Correlation coefficient P-value	0.017 0.932	0.355 0.059
	IL-23 Pre-treatment	Correlation coefficient P-value	-0.083 0.668	-0.283 0.138

periodontal status. Moreover, Duarte et al, only considered generalized periodontitis cases; while in this study, the samples were chosen from both localized and generalized periodontitis groups.

Rosalem et al, in 2011 found that in spite of a remarkable decrease in the concentration of IL-1β, IL-4, IL-8, and IFN-γ in the GCF after treatment, there was no significant statistical difference between the generalized aggressive and generalized chronic periodontitis [4]. Although in their study some other inflammatory cytokines were investigated, a similarity between the results is noticeable to some extent in the case of IL-23.

Ohyama et al, in 2009 reported that there was a higher concentration of IL-23 and IL-12 in patients with moderate-severe chronic periodontitis compared with healthy controls. However, the expression of IL-23 receptor was much higher in comparison with IL-12 receptor. They also found that the expression of IL-17 in the periodontal disease was elevated especially in areas of bone loss. They also found that in periodontal disease, the Th17 pathway is induced by IL-23 [9]. However, in their study, the effect of treatment on changes in concentration of IL-17 and IL-23 was not examined. According to the results of the current study, the remarkable decrease in the concentration of these two cytokines after treatment might represent the role of these two cytokines in periodontal pathogenesis.

Nevertheless, finding an inverse significant statistical correlation between the concentration of IL-17 before the treatment and clinical attachment loss in the aggressive periodontitis group in the present study shows that in chronic cases, the role of IL-17 in induction of tissue damage may be more critical than that in aggressive periodontitis.

Yu et al, in 2007 in the United States studied the protective role of IL-17 in bone resorption resulting from the effects of pathogens and observed that stimulation of IL-17RA Knock-Out mice with Porphyromonas gingivalis resulted in greater destruction of periodontal tissues which means that IL-17 can have a protective role against bone destruction [19]. The inverse

statistical correlation between IL-17 (before the treatment) and clinical attachment loss in the aggressive periodontitis cases in our study supported the findings of the above-mentioned study. It can be assumed that IL-17 plays a protective role with an inverse correlation with clinical attachment loss.

Beklen et al, in 2007 stated that the effect of IL-17 on induction of matrix metalloproteinases in fibroblasts was weaker than that of IL-1β and TNF-α and on this basis, they suggested IL-17 as a key regulatory cytokine [13]. There was an agreement between the results of the above-mentioned study and the present one about the inverse correlation of concentration of IL-17 before the treatment and clinical attachment loss in the aggressive periodontitis group.

Pradeep et al, in 2009 reported that the amount of IL-17 in GCF samples of patients with chronic periodontitis was near zero. They stated that IL-17 could not be considered as an index of periodontal disease development [22].

In this study, a decrease in the concentration of IL-17 was observed after the treatment in both aggressive and chronic periodontitis groups. Regarding to our results, in the chronic group, no significant statistical correlation was detected between the concentration of IL-17 and clinical indices, making it difficult to prove an observable role for this cytokine in induction of periodontal destruction. However, with regard to our pre-treatment observations, in primary examination of aggressive periodontitis group, an inverse statistical correlation between clinical attachment loss and IL-17 suggests a protective effect for IL-17.

Generally, we come to this conclusion that by administrating phase I periodontal treatment, a reduction in IL-17 and IL-23 pro-inflammatory markers, which are commonly considered as the key mediators of periodontitis pathogenesis, is observed. However, absence of a direct statistical relationship between the concentration of these two cytokines and the other clinical indices in the chronic periodontal group indicates that the reduction in GCF level of cytokines may not impact on the pathogenesis and manifestations of chronic

periodontitis. Presence of a direct statistical correlation between the concentration of IL-17 and IL-23 before the treatment in the chronic periodontal group confirms the role of IL-23 in induction of Th-17 cells and consequently, IL-17 production. However, because of limitations of sampling, this study was conducted by the convenience sampling method. Although it is a non-random sampling method, it is the most applicable and widely used method in clinical research. In future studies, we suggest broader investigations on the cytokines involved in periodontitis with a larger sample size and standard randomized sampling.

Conclusion

Considering the reduction of IL-17 and IL-23 levels in the GCF after nonsurgical periodontal therapy, these factors may play a role as mediators of the periodontitis pathogenesis. The direct statistical correlation between IL-23 and IL-17 levels before the treatment in the chronic periodontal group shows the possible role of IL-23 in inducing Th-17 cells and production of IL-17.

Regarding the inverse statistical correlation between the concentration of IL-17 before the treatment and clinical attachment loss in the aggressive periodontitis group, a probable protective role for IL-17 against aggressive periodontal disease can be considered.

Acknowledgements

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