BRIEF COMMUNICATION
Iran J Allergy Asthma Immunol
December 2021; 20(6):764-770.
Doi: 10.18502/ijaai.v20i6.8028

Evaluation of NLRP3 and IL-18 Levels after Periodontal Therapy

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Received: 10 May 2020; Received in revised form: 1 March 2021; Accepted: 14 March 2021

ABSTRACT

The progression of periodontitis depends on interactions between the periodontal pathogens and the host immune cytokines, including interleukin (IL)-1β and IL-18. Production of IL-1β is regulated by NOD-like receptors family pyrin domain containing 3 (NLRP3). This study aimed to evaluate the effect of periodontal treatment on the concentrations of IL-18 and NLRP3 in patients with chronic periodontitis.

In this experimental study, 18 patients with chronic periodontitis and a mean age of 46.2±8.95 years, were included. The gingival crevicular fluid (GCF) was collected at the beginning of the study, 4 weeks after non-surgical (phase I), and 4 weeks after surgical periodontal treatment. The levels of NLRP3 and IL-18 were measured; using an enzyme-linked immunosorbent assay. Pearson correlation test was used to analyze the concentration of NLRP3 and IL-18 before and after the treatments with CAL and PD.

There was a significant association between the level of NLRP3 and the mean values of PD and CAL before treatment. After each treatment phase, a significant decrease was observed in the NLRP3 level. There was no significant relationship between IL-18 and clinical parameters before and after periodontal treatments.

Given the possible association between the level of NLRP3 and clinical parameters, we suggest it as a possible indicator of inflammation in chronic periodontitis and an index for evaluating the treatment outcome.

Keywords: Chronic periodontitis; Interleukin-18; NLRP3 protein

INTRODUCTION

Periodontitis is a multifactorial inflammatory disease that destroys the supporting tissues of the teeth. This disease is induced by bacteria that activate the innate and adaptive immune system of the host.[1] The extent and progression of the periodontal disease depend on the interactions between the periodontal pathogens and the host immune system mediated by cytokines and chemokines produced by resident and migratory cells in the inflammation regions. From the immunological perspective, periodontitis is caused by a

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series of inflammatory reactions that include increased expression and production of inflammatory cytokines such as Interleukin (IL)-1β, tumor necrosis factor (TNF-α). IL-1β and IL-18 are inflammatory interleukins involved in the incidence of periodontitis and their imbalanced production results in tissue destruction.[2] Recently, it has been determined that the production of IL-1β is regulated by a family of proteins called NOD-like receptors (NLR). NLR family, pyrin domain containing 3 (NLRP3) is a member of this family.[3] NACHT LRR & PYD domains-containing protein 3 (NLRP3), known also as cryopyrin and PYRIN-containing Apaf1-like protein 1 (PYPAF1), is a member of the NLR family.[3] It has great potential for the identification of pathogens and danger-associated molecular patterns (DAMP) and mediates the immune responses against infection caused by pathogens and endogenous injuries.[4]

The inflammasome is a localized complex resulting from the aggregation of several proteins inside the cell cytoplasm. Inflammasome activation is an inflammatory process that induces the maturation of IL-1β and IL-18.[5] NLRP3, consisting of NLR, Apoptosis-associated Speck-like protein containing CARD (ASC), and Caspase-1, is the most important inflammasome.[4] Recent studies have shown that the main role of NLRP3 inflammasome is the regulation of IL-1β production in response to bacterial ligands such as lipopolysaccharides, peptidoglycans, and viral and bacterial RNA.[2] IL-18 is an inflammatory cytokine and a subset of interleukin-1 which is known as the interferon-gamma-inducing factor. IL-18 can differentiate Th1, which plays a role in immune response.[6]

According to studies, periodontal pathogens such as *P. gingivalis* and *A. actinomycetemcomitans* are involved in inflammasome activation,[7] there is a positive correlation between NALP3 and IL-18 expression in periodontitis and pocket depth (PD),[3] and a positive correlation between the concentration of IL-18 in gingival crevicular fluid (GCF) and clinical attachment level (CAL),[8] and its level increases with increasing the disease severity and decreases after treatment of patients with chronic periodontitis.[9]

Given the above-mentioned facts, and despite the studies carried out separately on each of these cytokines, no research has been conducted to investigate the effects of surgical and non-surgical treatments on the levels of NLRP3 and IL-18 in GCF. Therefore, the present study was designed to assess the effect of periodontal treatment on the levels of NLRP3 and IL-18 of GCF in patients visiting the periodontology department of the Shahed Dentistry Faculty in 2016-2017.

MATERIALS AND METHODS

This experimental study was performed on 18 patients (9 females and 9 males) with chronic periodontitis (chronic periodontitis is a destructive type of periodontal disease that are often characterized by slow progression),[10] who visited the department of periodontics, faculty of dentistry, Shahed University, in 2016-2017. Based on the Cochran formula and considering 10% possible loss; a total of 20 samples (sites) were estimated for each group. The study protocol was approved by the Ethics Committee of Shahed University and registered in the Iranian Registry of Clinical Trials (IR.shahed.REC.1396.40).

Inclusion Criteria was: the presence of at least 20 teeth, having moderate or severe chronic periodontitis (CAL≥3 mm, PD≥5 mm). PD is measured with a periodontal probe and is the distance from the gingival margin to the bottom of the probable sulcus. CAL measures the distance between attachment level and a fixed reference point.[11]

Subjects with systemic disease, fixed orthodontic appliance, history of allergy, having scaling and root planing or periodontal surgery during the last 6 months, taking antibiotics or anti-inflammatory drugs during the last three months, and use of tobacco were excluded from the study.

Before collecting GCF, the probing depth was measured with a Williams probe (Hu-Freddy-USA) in 4 areas around each tooth (mesiobuccal, mesiolingual, distobuccal, distolingual). To collect GCF, two areas were considered in each patient. These areas included periodontal PD≥5mm, CAL loss≥3 mm, with bleeding on probing (BOP). The selected areas were first washed by normal saline, then dried with a slow blast of air and isolated with a cotton roll. The supragingival plaque was removed with a curette without any contact with the gingiva. The perio-strips (GCF Smithtown, NY 11787 Oral flow) were placed inside the sulcus in proximal areas and left there for 30 seconds. The strips were then removed and carefully examined visually. The strips with blood, pus, saliva, or microbial plaque were discarded, and samples were recollected from the area. The perio-strips were then placed in air-tight

microtubes and stored in a -20°C freezer. Then nonsurgical treatment consisted of scaling and root planning was carried out with a piezoelectric ultrasonic unit (woodpecker).

After four weeks, the patients were recalled for re-sampling. Subsequently, the periodontal resection surgery was performed in all areas and sampling was repeated 4 weeks later from the same teeth sampled before in areas undergoing surgery. Oral Hygiene Instruction was performed in all treatment sessions. The levels of cytokines in GCF were measured through ELISA (450 nm, 620 references) using IL-18 (Interleukin-18 ELISA Kit, IBL, Germany, Cat No. BE51181) and NLRP3 (Human NALP3/NLRP3 Sandwich ELISA Kit, Lifespan Bioscience, USA, Cat No. LS-F17337) kits; based on the manufacturer's instructions.

Statistical Analysis

SPSS26 software was used for statistical analysis. The Kolmogorov Smirnov test was applied to evaluate the normal distribution of data. Wilcoxon test with Bonferroni adjustment was applied to pairwise comparing of concentrations of IL-18 and NLRP3 before and after each treatment. The Pearson correlation coefficient test was used to determine the correlation between IL-18 and NLRP3 levels before and after phase I and the surgical phase of treatment with CAL and PD. Statistical significance was set at $p \leq 0.05$.

RESULTS

This study was performed on 20 sites in 18 patients with moderate or severe chronic periodontitis (9 females and 9 males) with an average age of 46.5 years (35-58 years).

Descriptive statistical parameters of PD, CAL, and levels of IL-18 and NLRP3 before treatment and after phase I (nonsurgical) and surgical treatment are presented in Table 1.

Figure 1 is shown Boxplot for NLRP3, which is classified by levels and presents information on the variability or dispersion of the data.

Kruskal Wallis Test was used for comparison of NLRP3 Levels and the effect size for this test was calculated 0.192, which is large.

The hypothesis of normality of NLRP3 level for each treatment was rejected and there is a significant difference between levels of NLRPS (p=0.001). Pairwise comparing of NLRP3 concentration showed the difference between nonsurgical treatment and after surgery wasn't significant (p=0.39) but there was a significant difference between before treatment with nonsurgical treatment (p=0.01) and after surgery (0.001).

Surgical and non-surgical periodontal treatments have no significant effect on IL-18 levels in GCF (p>0.05). Each treatment phase significantly reduced the NLRP3 level.

Table 1. Descriptive statistical parameters of probing depth (PD), clinical attachment level (CAL), and levels of interleukin (IL)-18 and NLR family pyrin domain containing 3 (NLRP3) before treatment and after non-surgical and surgical treatment

Variables	Number	Minimum	Maximum	Mean	SD	Unit
PD	20	6	10	7.55	1.356	mm
CAL	20	6	11	7.70	1.593	mm
IL-18 before treatment	20	0.08	0.40	0.1410	0.06865	ng/mL
IL-18 after scaling and root planning	20	0.01	0.91	0.1439	0.18542	ng/mL
IL-18 after surgery	20	0.09	0.30	0.1354	0.05082	ng/mL
NLRP3 before treatment	20	0.000	0.600	0.18620	0.143156	ng/mL
NLRP3 after scaling and root planning	20	0.000	0.410	0.07840	0.107931	ng/mL
NLRP3 after surgery	20	0.000	0.310	0.04400	0.085864	ng/mL

The results of the Pearson test showed no significant correlation between IL-18 and NLRP3 at any time point, especially before treatment (p>0.8).

The Pearson correlation coefficient test was used to determine the correlation between the levels of IL-18 and clinical parameters before and after treatments; there was no significant correlation neither in PD nor in CAL (p<0.4).

The correlation coefficient of NLRP3 level before treatment with PD and CAL was also determined and a statistically significant correlation was found in both cases (p<0.001). Confidence interval 95% for The correlation coefficient of NLRP3 level before treatment with CAL is 0.380 to 0.750.

According to Table 2, there was a significant correlation between NLRP3 levels and PD (p<0.001) with CI 95% (0.400, 0.880).

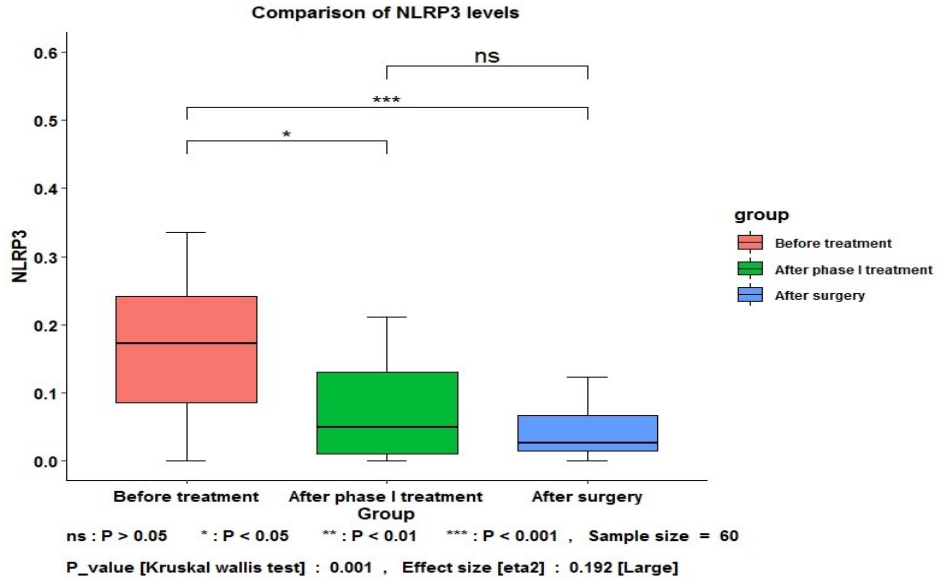

Figure 1. Comparison of NLR family pyrin domain containing 3 (NLRP3) level before treatment, after non-surgical treatment, and after surgery

Table 2. Pearson correlation coefficient between NLR family pyrin domain containing 3 (NLRP3) level before treatment and probing depth (PD)

		PD	NLRP3 level before treatment
PD	Pearson Correlation	1	0.716**
	Sig. (2-tailed)		**0.000**
	N	20	20
NLRP3 level before treatment	Pearson Correlation	0.716**	1
	Sig. (2-tailed)	**0.000**	
	N	20	20

**correlation is significant at level 0.01

DISCUSSION

The results of the study showed that the NLRP3 level in GCF in patients with chronic periodontitis was significantly higher before treatment, and was significantly reduced after nonsurgical periodontal treatment. The significant correlation between NLRP3 level before treatment with CAL and PD which was seen in this study, suggests a correlation between NLRP3 and periodontal tissue degradation. However, there was no significant difference in IL-18 before and after treatments, despite its decrease and no significant relationship existed between IL-18 level and clinical parameters.

Guzmán et al reported that salivary NLRP3 and IL-1β levels in patients with chronic periodontitis are significantly higher than in healthy subjects.[12] Park et al, stated that *P. gingivalis* can induce the production of IL-1β and the NLRP3-dependent death of muscle and human phagocytes.[13] Montenegro et al, also reported that dental calculus can result in IL-1β production and secretion from phagocytes with the same mechanism (NLRP3-dependent).[14] Xue et al, observed a higher expression of NLRP3 in gingival tissues in patients with chronic periodontitis compared with healthy gum tissue and stated that they may contribute to the progression of periodontal inflammation.[14]

Despite the study of different samples and the fact that the above studies do not address the effect of treatment, there is some similarity between the results of the above studies and the findings of the present research; because in our study, NLRP3 had the highest level in GCF of patients with chronic periodontitis before treatment. Belibasakis and Johansson stated that *A. actinomycetemcomitans* can increase NLRP3.[15] Since *A. actinomycetemcomitans* is an important periodontal pathogen, it can be stated that there is no contradiction between the results. Qun-Guang Mei et al, concluded that the highly expressed NLRP3 inflammasome in periodontal tissues of patients with periodontitis can intensify the inflammatory response and alveolar bone resorption.[16]

Regarding the relationship between IL-18 and chronic periodontitis, Türkoğlu et al, reported that IL-18 is directly related to periodontitis and is considered as a part of the host's innate defense response to periodontopathic pathogens.[17] Sánchez-Hernández et al, stated that serum levels of IL-18 are significantly higher in patients with chronic periodontitis than in patients with aggressive periodontitis and healthy subjects.[18] In contrast, Ozçaka et al, stated that the salivary levels of IL-18 in chronic periodontitis are significantly higher than that of the healthy group, but there is no significant difference in the serum levels of this interleukin between the two groups.[19] As can be seen, the reason for the difference between the results can be justified as follows; in our study, the level of IL-18 in GCF was not evaluated in healthy individuals, and if compared with healthy subjects, we may also find a significant statistical difference in the level of IL-18 (before treatment) between patients with chronic periodontitis and healthy subjects. Even in studies that show an increase in IL-18 level, this increase has not been reported for all types of the studied samples. Yee et al, reported that *P. gingivalis* stimulates the production and secretion of active forms of IL-18 from human THP-1 monocytes, which can lead to the progression of periodontitis.[20] The present study was performed on human periodontal patient samples, not *in vitro*, which could justify the difference in the results. It should be noted, however, that the above study was performed only on the effect of *P. gingivalis* in cell culture; therefore the conclusion regarding the effect of IL-18 on the progression of periodontitis seems not reasonable. Banu et al, stated that the salivary levels of IL-18 and TLR4 were significantly higher in patients with chronic periodontitis than in healthy subjects and found a significant correlation between their concentration and clinical parameters.[21]

This lack of similarity comes from the fact that the above research has been conducted on serum and saliva but in our study, we focused on GCF.

Regarding the effect of periodontal treatment on the level of IL-18, Mahajani et al, reported that patients with chronic periodontitis have significantly a higher level of IL-18 in GCF than healthy subjects which reduced significantly 3 and 6 weeks after non-surgical periodontal treatment.[22] In the present study, the level of IL-18 was also reduced after surgical treatment, although the reduction was not significant, after the Phase I treatment, a slight increase was observed, which can be attributed to the time of the evaluations. It should be also noted that, in addition to several types of bacteria, many other factors play a role in the occurrence of periodontitis, such as host genetics, which has a crucial role in the onset of inflammatory

responses. The above study was performed on Indians in Central Maharashtra, which may have racial differences; so that de Campos et al did not find also a significant statistical difference in the level of IL-18 in Brazilian chronic periodontitis patients.[23]

According to the findings of this study, this hypothesis can be suggested that IL-18 may not be an appropriate indicator for determining the inflammatory status of periodontal tissues, but NLRP3 may be considered as an indicator of inflammation in chronic periodontitis. Given the possible association between the level of NLRP3 and clinical parameters, we can suggest it as a possible indicator for predicting the condition of periodontal tissue degeneration and an appropriate index for evaluating the treatment performed on patients. However, further research is needed to prove this hypothesis.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

ACKNOWLEDGEMENTS

This research has been supported by a grant from the Research Council of the Shahed University, Tehran, Iran.

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