

## The Influence of Smoking on IL-17 Cytokine in Chronic Periodontitis Patients

Eric Sulistio<sup>1</sup>, Sri Lelyati C. Masulili<sup>2\*</sup>, Robert Lessang<sup>2</sup>, Elza Ibrahim Auerkari<sup>3</sup>

1. Department of Periodontics Residency Program, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.

2. Department of Periodontics, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.

3. Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.

### Abstract

Interleukin-17 is a cytokine derived from T cells. This cytokine has a role in beginning and continuing a pro-inflammatory sensitivity and the development of periodontal disease.

To investigate the effect of IL-17 levels in the gingival crevicular fluid of smokers compared with the non-smokers with chronic periodontitis. GCF samples were taken from the deepest pocket affected by periodontal disease (attachment loss  $\geq$  3mm) from 14 subjects of smokers and 11 subjects of non-smokers.

The examination of IL-17 levels using ELISA. There were no significant differences in total IL-17 levels in GCF between smokers and non-smokers with chronic periodontitis and there was no significant change in IL-17 levels in GCF related to the amount of cigarette consumption. Smoking did not significantly affect total levels of IL-17 in chronic periodontitis.

**Clinical article (J Int Dent Med Res 2019; 12(1): 199-202)**

**Keywords:** Chronic periodontitis, IL-17, GCF, Smoking.

**Received date:** 15 August 2018

**Accept date:** 20 September 2018

### Introduction

Chronic periodontitis is a multifactorial disease that involving microorganism and host response that causes periodontal tissue damage such as attachment loss, gingival recession, and bone loss.<sup>1-3</sup> Several methods for diagnosis were used to find specific microorganisms and evaluate the nature of host responses in serum, gingival crevicular fluid (GCF), and/or saliva. Inflammatory products are abundant in the GCF so that periodontal cells are affected by factors such as cytokines, prostaglandins, and pathogenic bacteria. The factors that play main role in the development of periodontitis are cytokines from monocytes, macrophages and polymorphonuclear neutrophils (PMNs). They act as an important part of defenses against bacterial lipopolysaccharides and help in signaling active macrophages and differentiating between CD8 and CD4 cells.<sup>1</sup>

Homeostasis between CD8 and CD4 (T-helper1 and T-helper2, or Th1 and Th2 cells) is a central key in the development of immunity in

periodontal diseases. Products from Th1 and Th2 produce responses at the cellular and hemoral levels. In primary periodontal lesions, Th1 cells slightly increase temporarily in advanced lesions affecting Th2 cells.<sup>2</sup> This IL-17 cytokine has biological activity with fibroblasts, endothelial cells to produce other inflammatory cytokines and chemokines. IL-17 secret cells that have been shown to affect inflammatory lesions in patients such as autoimmune diseases including multiple sclerosis, rheumatoid arthritis, psoriasis, inflammatory bowel disease, type 1 DM, and periodontitis.<sup>2,3</sup> Several studies have reported the degree of IL-17 levels in patients with acute periodontitis.<sup>4,5</sup>

Many studies showed that smoking was one of the most important local factors that affect risk factors. Smoking was not only closely related to risk but also the prognosis of periodontitis. Smokers were thought to be associated with advanced periodontitis and aggressive periodontitis which show a large degree of bone loss.<sup>5</sup> The differences between smokers and non-smokers in periodontal treatment were that smokers had a poor response and having persistent periodontitis more frequently. Smoking habits affected body responses such as neutrophil/monocyte activities, vascular function, antibody production, expression of adhesion molecule, cytokine and the release of the inflammatory mediator. Smoking could activate complex inflammatory factors that produce

#### \*Corresponding author:

Sri Lelyati

Department of Periodontics,  
Faculty of Dentistry, Universitas Indonesia,  
Jakarta, Indonesia.

E-mail: srilelyati@yahoo.com

such as strong cytokines and chemokines, which contribute to the development of periodontal disease.<sup>6-12</sup> The existing studies showed that there were still differences in the results. Considering the different results and lack of sufficient studies, especially in Indonesia, the aim of this study was to compare the levels of IL-17 in the GCF of smoking and non-smoking patients with chronic periodontitis and investigating the extent of the effect of smoking on IL-17 levels in patients with chronic periodontitis.

## Methods

**Patients.** The study group consisted of 76 samples from 25 male adult patients, 35–65 ( $47.24 \pm 9.87$ ) years old, with chronic periodontitis, including 14 smokers and 11 non-smokers. They were the patients of the Dental Hospital at the Faculty of Dentistry, Universitas Indonesia (FKG UI) and from Depok. The inclusion criteria included the smokers and non-smokers patients had moderate to advanced periodontitis, i.e. at least five to six teeth had probing depth (PD)  $\geq 5$  mm and clinical attachment loss (CAL)  $\geq 3$  mm and had not received any dental treatment for the last six months. The subjects have not the systemic disease and have not received antibiotics in the last 3-month. The smokers were further grouped into consumption cigarettes. (light = 10 cigarettes a day, moderate = 11-20 cigarettes a day, and heavy = more than 20 cigarettes a day)

**Clinical measurements.** The periodontal clinical examination consists of pocket depth (PD), bleeding on probing (BOP) and clinical attachment loss (CAL) were evaluated and measured on six sides of each tooth (mesiobuccal, mild-buccal, distobuccal, mesiolingual, distolingual, and mid-lingual). A periodontal probe UNC-15 was used for attachment level and PDs (Osung, Korea).

**Collection of data and GCF.** Data collection of questionnaires consists of smoking habits, periodontal tissue examination, and GCF collection were conducted from May to June 2018 in Depok and Dental Hospital of FKG UI. Samples were taken from two sites in the affected sites with criteria (PD  $\geq 5$ mm, CAL  $\geq 3$ mm) from 14 smoking patients with periodontal

disease, and from 11 non-smoking patients with periodontal disease.

The tooth was dried, a supragingival plaque was cleaned with curettes and avoid bleeding in the gingiva. The crevicular sites were dried with a cotton pellet or gently with an air from the three-way syringe. GCF was taken with paper points. Paper points were inserted into the sulcus/pocket until moist was seen in paper points and left in place for 30 seconds. Paper points contaminated by saliva or blood were thrown away from the sample group. After 30 seconds, paper points were taken and placed in PBS. GCF was processed and precipitated with centrifugation to get the extract, then analyzed immediately (within 2 hours) or aliquot stored at  $-20^{\circ}\text{C}$  until further analysis, avoiding multiple freeze-thaw cycles in the process.

### **Quantification of cytokine IL-17 in GCF.**

The IL-17 concentration was measured in gingival crevicular fluid samples using the IL-17 ELISA kit (MyBioSource, San Diego, CA, USA) according to the manufacturer's instructions.

**Data analysis.** The level of cytokine IL-17 in the two groups was shown as a mean  $\pm$  standard deviation. The quantity of level IL-17 was calculated between two groups using an unpaired Mann-Whitney-test. The significance data for each group was valued using the Kruskal-Wallis test.

## Results

Tables 1 showed the mean levels of cytokine in GCF. The levels of cytokine in gingival crevicular fluid were significantly different between smokers and non-smokers. The mean age of smokers was about six years higher than that of non-smokers. Table 2 showed the mean levels of IL-17 in GCF with chronic periodontitis disease. The levels of IL-17 in gingival crevicular fluid were not significantly different between smokers and non-smokers with chronic periodontitis.

Also, no significant differences were observed in IL-17 levels in total consumption of cigarettes between patients smoking 11-20 cigarettes per day and those smoking more than 20 cigarettes per day (Table 3).

Variable	Smokers (n=59)		Non-smokers (n=17)	
	Mean (SD)	Min-Max	Mean (SD)	Min-Max
IL-17(pg/mL)	1.61 (0.16)	1.33-2.08	1.39 (0.65)	0.74-2.95
Age (years)	47.24 (9.87)	35-65	41.10 (2.96)	37-46

**Table 1.** IL-17 Levels in GCF of Smokers and Non-Smokers

Subjects	n	Level of IL-17 (pg/mL)		p-value*
		Mean (SD)	Median (min-max)	
Smokers	59	1.61 (0.16)	1.58 (1.33-2.08)	0.713
Non-Smokers	10	1.75 (0.63)	1.54 (1.07-2.95)	

\*Kruskal-Wallis test

**Table 2.** IL-17 in GCF of Smokers and Non-Smokers with Chronic Periodontitis

Cigarette Consumption	n	Level of IL-17 (pg/mL)		p-value
		Mean (SD)	Median (min-max)	
<11 cigarettes/day	2	1.58	1.58	0.988
11-20 cigarettes/day	33	1.62 (0.17)	1.58 (1.41-2.08)	
>20 cigarettes/day	24	1.61 (0.16)	1.58 (1.33-1.98)	

Kruskal-Wallis test

**Table 3.** IL-17 Levels of Smokers According to Cigarette Consumption

## Discussion

Periodontitis is a disease that causes inflammation of the supporting tissues of teeth that are due to bacteria of specific microorganisms such as anaerobic Gram-negative bacteria. Periodontitis can cause progressive damage to the all supportive tissue around the teeth like ligament periodontal, cementum, gingiva and alveolar bone then eventually become pocket and some recession. Several studies in recent years have confirmed the main part of cytokines in the pathogenesis of chronic periodontitis which can be ascertained to be influenced by certain environmental factors such as smoking.

In this study, IL-17 GCF levels were compared between smoking and non-smoking patients with chronic periodontitis. This is the first study to investigate the possible effects of smoking on influencing levels IL-17 on the GCF in smoking and non-smoking patients with chronic periodontitis in Indonesia. Smokers have an incidence four times more likely to have advanced periodontitis than non-smokers. According to Vernal et al., IL-17 levels in the GCF of patients with periodontitis were higher compared to healthy control patients and IL-17 played a role in the

pathogenesis of chronic periodontitis. This can occur because the role of IL-17 affects the immunity and contributes to making clinical disease, and also the identification and characteristics of other molecules play a role.<sup>1-3</sup> The presence of concentrations of IL-17 is a consequence of the early stages of gingival inflammation, but it does not cause periodontitis lesions.

According to this study, our data showed that both smoking and periodontal inflammation in chronic periodontitis did not affect GCF levels in patients. Smoker and non-smoker patients with chronic periodontitis seem to have the same IL-17 concentrations. It is possible because IL-17 is an important component of periodontal tissue inflammation, which facilitates a movement to a 'pathogenic' microbial community.<sup>2</sup>

The high amount of IL-17 production could affect the pathogenic role in periodontitis. IL-17 had an effect to cause increased inflammation through excessive recruitment of neutrophils, by increasing the production of the pro-inflammatory cytokine.<sup>2</sup> In smokers, it was suspected that the production of pro-inflammatory biomarkers will be suppressed, but these mediators were sufficient to make pathogenesis of periodontal.

The number of patients who participated was not in a greater amount, especially the group of patients who did not smoke. It could be an influencing factor to get significant results between the smoker and non-smoker with chronic periodontitis. The drawback of this study is that patient report self-history, smoking history and the total amount of cigarette consumption is only based on the patient's own memory. And also in this study, the smoking/non-smoking group questionnaire was only based on the patient's own knowledge. In addition, patients in the non-smoker group may have become passive smokers from family members who are still active smokers and it may affect biochemical substances since there is an assumption that smoke which was inhaled can activate proinflammatory cytokines. And we know that passive smokers can also have negative effects in systemic conditions as in active smokers. These factors need to be carried out a further research to investigate whether the effect of these factors can influence the results of the research conducted.

## Conclusions

The results showed that smoking and the total amount of cigarette consumption were not associated with increased levels of inflammatory IL-17 in subjects with chronic periodontitis.

## Acknowledgement

The publication of this manuscript is supported by Universitas Indonesia.

## Declaration of Interest

The authors report no conflict of interest.

## References

1. Rohaninasab M, Sattari M, Abedi H, Zarenejad N. The Effect of Periodontal Therapy on IL-17 and IL-23 in Gingival Crevicular Fluid (GCF) of Patients with Severe Periodontitis. *Caspian J Dent Res.* 2013;2(1):32-8.
2. Abusleme L, Moutsopoulos NM. IL-17: Overview and Role in Oral Immunity and Microbiome. *Oral Dis.* 2017;23(7):854-65.
3. Zenobia C, Hajishengallis G. Basic Biology and Role of Interleukin-17 in Immunity and Inflammation. *Periodontol 2000.* 2015;69(1):142-59.
4. Takashi A. Studies on the Role of Interleukin-17 in the Progression of Periodontitis. *J Japanese Asso Periodontol.* 2002;44:281-91.
5. Marina T, Ioanna T, Maria M, Lazaros T, Antonios K. The Effect of Smoking on Components of Gingival Crevicular Fluid in Patients with Periodontal Disease. *OHDM.* 2015;14(6):1-5.
6. Al-Ghurabi BH. Impact of Smoking on The IL-1B, IL-8, IL-10, IL-17 and TNF- $\alpha$  Production in Chronic Periodontitis Patients. *J Asian Sci Res.* 2013;3(5):462-70.
7. Chahal GS, Chhina K, Chhabra V, Chahal A. Smoking and its Effect on Periodontium – Revisited. *Indian J Dent Sci.* 2017;9:44-51.
8. Gümüş P, Buduneli E, Bıyıkoğlu B, et al. Gingival Crevicular Fluid, Serum Levels of Receptor Activator of Nuclear Factor-Kappa B Ligand, Osteoprotegerin, Interleukin-17 in Rheumatoid Arthritis and Osteoporosis Patients With Periodontal Disease. *J Periodontol.* 2013;84(11):1-14.
9. Calsina G, Ramon JM, Echeverria J. Effects of Smoking on Periodontal Tissues. *J Clin Periodontol.* 2002;29(8):771-6.
10. Saraiva AM, Alves e Silva MR, Correia Silva Jde F, et al. Evaluation of IL17A Expression And Of IL17A, IL17F And IL23R Gene Polymorphisms In Brazilian Individuals With Periodontitis. *Hum Immunol.* 2013;74(2):207-14.
11. Liu YC, Lerner UH, Teng YT. Cytokine Responses Against Periodontal Infection: Protective and Destructive Roles. *Periodontol 2000.* 2010;52(1):163-206.
12. MyBioSource. Human IL-17 ELISA Kit. Available at: "[https://www.mybiosource.com/prods/ELISA-Kit/Human/IL-17/datasheet.php?products\\_id=355268#QLREF](https://www.mybiosource.com/prods/ELISA-Kit/Human/IL-17/datasheet.php?products_id=355268#QLREF)".