

# THE RELATIONSHIP BETWEEN INTERLEUKIN-18 LEVEL IN SMOKERS AND CHRONIC PERIODONTITIS: RADIOGRAPHIC OVERVIEW OF POSTERIOR MANDIBULAR TEETH

F.X. Andi Wiyanto<sup>1</sup>, Sri Lelyati C. Masulili<sup>2</sup>, Elza Ibrahim Auerkari<sup>3</sup>, Fatimah Maria Tadjoeidin<sup>2</sup>

<sup>1</sup>Periodontics Residency Program, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia

<sup>2</sup>Department of Periodontics, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia

<sup>3</sup>Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia

## ABSTRACT

**INTRODUCTION:** Tobacco smoking is a very common addiction worldwide and an environmental factor predisposing to periodontal disease. The role of cytokines and chemokines in chronic periodontitis pathogenesis has been previously confirmed. Evaluating serum cytokines and chemokines is essential in determining inflammatory responses in periodontitis patients. Previous findings imply a close relationship between elevated cytokine interleukin-18 (IL-18) levels and chronic periodontitis pathogenesis in smokers; however, the usefulness of IL-18 as an inflammatory marker in gingivitis remains unclear. The aim of the study was to analyze IL-18 levels in smokers with chronic periodontitis.

**OBJECTIVES:** The purpose of this study was to analyze the relevance of IL-18 levels to the severity of chronic periodontitis in smokers in terms of the radiographic features of mandibular posterior teeth.

**MATERIAL AND METHODS:** A cross-sectional study of 76 male subjects aged 19-34 years with chronic periodontitis in Depok, Indonesia was performed. Clinical data (OHIS, pockets, CAL), smoking status, and IL-18 samples were collected; samples were detected using ELISA.

**RESULTS:** IL-18 levels in smokers with moderate periodontitis were higher than in those with mild periodontitis. However, the results showed that the differences in IL-18 levels were not significant based on daily cigarette consumption, and no significant correlation was revealed regarding IL-18 concentrations in smokers based on smoking duration. The correlation test results demonstrated a significant relationship between periodontitis severity and the number of cigarettes consumed per day, but no significant correlation between periodontitis severity in smokers and smoking duration.

**CONCLUSIONS:** IL-18 levels in saliva and gingival crevicular fluid can be used as a predictable biomarker for periodontal disease progression, but cannot be used to determine the patients' smoking habits. Further studies are required to confirm the relationship between IL-18 and chronic periodontitis in smokers.

**KEY WORDS:** interleukin-18, chronic periodontitis, smokers.

J Stoma 2018; 71, 3: 254-258

DOI: <https://doi.org/10.5114/jos.2018.80648>

**JOURNAL OF  
STOMATOLOGY**  
CZASOPISMO STOMATOLOGICZNE

OFFICIAL JOURNAL OF THE POLISH DENTAL SOCIETY | ORGAN PUBLIKACJI TOWARZYSTWA STOMATOLOGICZNEGO



**ADDRESS FOR CORRESPONDENCE:** Sri Lelyati C. Masulili,  
Department of Periodontics, Faculty of Dentistry, Universitas Indonesia,  
Jakarta, Indonesia, phone: +62213911502, fax: +62213911502,  
e-mail: [srilelyati@yahoo.com](mailto:srilelyati@yahoo.com)

## INTRODUCTION

Smoking is one of the most common addictions in the world today. The World Health Organization estimates that there are approximately 1,100 million tobacco smokers worldwide [1]. Smoking has been implicated as an etiological agent for various chronic diseases, including infections, cancers, heart diseases, and chronic lung diseases, which together are the leading causes of morbidity and mortality in the world [2]. Cigarette smoking has been associated with periodontal disease for the past 20 years [3], but smoking alone does not cause periodontal disease. Periodontal disease is caused by bacterial plaque that accumulates underneath gingival tissues and becomes infected, initiating bone loss around the teeth. Once periodontal disease starts, smokers lose bone at a faster rate than nonsmokers due to the effect of nicotine on the circulatory system, which in turn reduces blood flow and oxygen intake by hemoglobin, hindering the body's ability to fight the infection and bone resorption [4].

Several studies have confirmed the role of cytokines and chemokines in the pathogenesis of chronic periodontitis. The secretion of cytokines and chemokines can be influenced by certain environmental factors, of which tobacco smoking is one of the greatest influences. One such host factor is interleukin (IL)-18, a multifunctional proinflammatory cytokine from the IL-1 family. It was initially identified as an interferon- $\gamma$  (IFN- $\gamma$ ) inducer and subsequently associated with obesity, atherosclerosis, insulin resistance, glucose intolerance, and cardiovascular diseases. In addition, high levels of IL-18 can be considered as predictive for type 2 diabetes progression [5]. Recently, IL-18 was reported to be higher in both gingival tissue and gingival crevicular fluid in patients with periodontitis than in healthy subjects [6], suggesting that excessive IL-18 in gingival tissue could be a key stimulator of periodontal disease.

Different results were found by Boronat-Catalá *et al.* In 2 of the 3 studies that analyzed IL-18 using the enzyme-linked immunosorbent assay (ELISA), the authors found lower IL-18 levels in the healthy and high-risk patient groups with periodontitis and no difference in the other study groups. No study has linked IL-18 with chronic periodontitis in smokers. Therefore, there is no clear consensus on the utility of IL-18 as an inflammatory marker of gingivitis; thus, further research is needed to determine a relationship between IL-18 and chronic periodontitis in smokers [7].

The severity of chronic periodontitis can be detected by radiographic features. The posterior mandible is the region with the majority of periodontal abnormalities and is in position for manipulation of radiographic imaging as it is not at the angle of the jaw [8]. There are few reports on the role of IL-18 in the treatment of chronic periodontitis in smokers. No similar research has examined the severity of chronic periodontitis via

radiographic features. Chronic periodontitis is often found in the mandibular posterior teeth [9, 10].

## OBJECTIVES

The purpose of this study was to analyze the relevance of IL-18 levels to the severity of chronic periodontitis in smokers in terms of the radiographic features of mandibular posterior teeth.

## MATERIAL AND METHODS

### PATIENT POPULATION

A cross-sectional study was performed in 76 smokers, ranging in age between 19 and 34 years, who were selected based on the previously established inclusion and exclusion criteria. All study subjects were men working as police officers at Depok. The initial research phase was conducted at the dentistry department at Depok in April 2018. All subjects were asked to fill out a questionnaire that included questions on smoking habits, and samples of gingival crevicular fluid were collected from them to determine IL-18 levels. Next, ELISA techniques were used to analyze the samples in the Integrated Laboratory of the Faculty of Medicine, Universitas Indonesia. Radiographic examinations were conducted to determine the degree of severity of periodontal status in April 2018 at the Radiology Department of Bhayangkara Kelapa Dua Hospital, Depok, using the periapical radiographic technique.

### INCLUSION CRITERIA

The study subjects included adults aged 19-34 years, with a history of chronic periodontitis and smoking who were willing to be a research sample and provide signed informed consent.

### EXCLUSION CRITERIA

Exclusion criteria included a history of aggressive periodontal disease, systemic disease, not smoking, no periodontal treatment in the last 3 months, and use of antibiotics in the past month.

### SAMPLE COLLECTION

Gingival crevicular fluid samples were collected from periodontal pockets in patients with chronic periodontitis and from gingival sulcus in periodontally healthy patients by placing paper points in the sulcus or pocket for 30 seconds. Supragingival plaque was cleaned from the oral and interproximal surfaces of the teeth, dried

**TABLE 1.** Distribution of subjects by age

Age (years)	N (76)	%
< 20	4	5.26
21-25	58	76.32
26-30	7	9.21
31-34	7	9.21

**TABLE 2.** Distribution of subjects by smoking habit

Subjects	N (76)	%
Duration of smoking		
< 10 years	65	85.52
10-20 years	11	14.48
> 20 years	0	0
Total cigarette consumption per day		
Light smokers	24	31.58
Moderate smokers	29	38.16
Heavy smokers	13	30.26

\*Light smokers smoked ≤ 10 cigarettes, moderate smokers smoked between half and 1 pack per day, and heavy smokers smoked between 1 and 2 packs per day [10].  
\*1 pack = 20 cigarettes [10].

gently with air, and kept dry with gauze. Samples of filter Periopaper strips were stored in Eppendorf tubes at -20°C for later immunological analysis.

## ELISA TO MEASURE IL-18

The IL-18 concentration was measured in gingival crevicular fluid samples using an IL-18 ELISA kit (MyBioSource, San Diego, CA, USA) according to the manufacturer's instructions.

## STATISTICAL ANALYSIS

Next, numerical descriptive data were analyzed using SPSS Statistics software version 20.0. Data analysis in this research used descriptive statistics and statistical hypotheses to analyze whether there are differences in IL-18 levels between groups of research samples. The average comparison test in this study used one-way analysis of variance and post hoc analysis with the *t* test.

## RESULTS

### SUBJECT DISTRIBUTION

The distribution of subjects by age is shown in Table 1. The data show that most subjects (as many as 58 people [76.32%]) ranged in age between 21 and 25 years. Four subjects (5.26%) were aged < 20 years.

**TABLE 3.** Distribution of IL-18 levels by degree of periodontitis and total cigarette consumption per day

Variables	N (76)	IL-18 levels (pg/ml)	
		Mean (SD)	Min-Max
Degree of periodontitis			
Mild	49	6.22 (3.30)	2.83-20.23
Moderate	27	8.10 (4.21)	2.00-18.88
Total cigarette consumption per day			
Light smokers	24	6.28 (3.36)	2.00-18.88
Moderate smokers	39	7.23 (4.24)	2.69-20.23
Heavy smokers	13	6.98 (2.71)	3.27-10.61

\*Light smokers smoked ≤ 10 cigarettes, moderate smokers smoked between half and 1 pack per day, and heavy smokers smoked between 1 and 2 packs per day [10].  
\*1 pack = 20 cigarettes [10].

Subjects were divided based on their smoking habits calculated by smoking duration and total cigarette consumption per day (Table 2). Subjects who smoked 1-10 years had the largest distribution (65 [85.52%]) and subjects who smoked for 10-20 years had a smaller distribution (11 [14.48%]). Overall, 29 subjects (38.16%) smoked 11-20 cigarettes per day, 24 (31.58%) smoked ≤ 10 cigarettes per day, and 13 (30.26%) smoked > 20 cigarettes per day.

## UNIVARIATE ANALYSIS

Table 3 shows the data distribution results for the current study. Subjects with moderate periodontitis had a higher mean value of IL-18 (8.10 ± 4.21 pg/ml) than those with mild periodontitis (6.22 ± 3.30 pg/ml). IL-18 levels in moderate smokers were higher (7.23 ± 4.24 pg/ml) than those in heavy smokers (6.98 ± 2.71 pg/ml) and light smokers (6.28 ± 3.36 pg/ml).

## BIVARIATE ANALYSIS

The data normality test was performed to determine the distribution of data for IL-18 with regard to the degree of periodontitis, the number of cigarettes smoked per day, and the smoking duration in the study sample. Data are presented in Table 4.

The normality test of data using the Shapiro-Wilk test was done because each group of subjects had a total sample of < 50 participants. The results of the normality test of data indicate that each variable has abnormal data normality distribution.

Differences in IL-18 levels in smokers based on the degree of periodontitis were analyzed using the Mann-Whitney test. Table 5 shows higher IL-18 levels in smokers with moderate versus mild periodontitis (*p* < 0.05).

A comparative analysis of IL-18 levels according to daily cigarette consumption was conducted using the Kruskal-Wallis nonparametric comparative test

**TABLE 4.** Data normality test results

Variables	IL-18 levels
Degree of periodontitis	
Mild	0.001
Moderate	0.067*
Total cigarette consumption per day	
Light smokers	0.001
Moderate smokers	0.001
Heavy smokers	0.07*
Smoking duration	0.001

Shapiro-Wilk test, \* $p \geq 0.05$  normal data distribution.  
 \*Light smokers smoked  $\leq 10$  cigarettes, moderate smokers smoked between half and 1 pack, and heavy smokers smoked between 1 and 2 packs per day [10]. 1 pack = 20 cigarettes [10].

**TABLE 5.** Comparative analysis of IL-18 levels in smokers based on periodontitis degree

Periodontitis degree	IL-18 levels (pg/ml)		<i>p</i>
	Mean (SD)	Median (Min-Max)	
Mild	6.22 (3.30)	5.36 (2.83-20.23)	0.024*
Moderate	8.10 (4.21)	7.03 (2.00-18.88)	

Mann-Whitney test, \* $p < 0.05$  indicates significant difference.

(Table 6). The results showed that the differences in IL-18 levels were not significant based on daily cigarette consumption.

A correlation analysis of IL-18 concentrations in smokers based on the smoking duration using the Spearman rank correlation coefficient did not show a significant correlation ( $p > 0.05$ ), as shown in Table 7.

As many as 30 subjects (76.9%) were considered as moderate smokers with mild periodontitis, whereas at least 4 (30.8%) were heavy smokers with moderate periodontitis. The association between the degree of periodontitis and the number of cigarettes consumed per day was analyzed using the Pearson chi-squared test (Table 8). The correlation test results revealed a significant relationship ( $p < 0.05$ ) between the degree of periodontitis and the number of cigarettes consumed per day.

The correlation test results (Table 8) showed a significant relationship between periodontitis severity and the number of cigarettes consumed per day, but different results are shown in Table 9. Table 9 revealed that there is no significant correlation between the severity of periodontitis in smokers and the smoking duration.

## DISCUSSION

Cytokines play a crucial role in inflammatory and immune responses during periodontal disease progression. Monitoring cytokine production may be particularly useful in diagnosing periodontal disease initiation and pro-

**TABLE 6.** Comparative analysis of IL-18 in smokers based on cigarette consumption per day

Total cigarette consumption per day	IL-18 levels (pg/ml)		<i>p</i>
	Mean (SD)	Median (Min-Max)	
Light smokers	6.28 (3.36)	5.70 (2.00-18.88)	0.709
Moderate smokers	7.23 (4.24)	7.23 (2.69-20.23)	
Heavy smokers	6.98 (2.71)	6.57 (3.27-10.61)	

Kruskal-Wallis test, \* $p < 0.05$  indicates significant difference.

**TABLE 7.** Correlation analysis of IL-18 to smoking duration

Smoking	IL-18 levels (pg/ml)	
	<i>r</i>	<i>p</i>
Smoking duration	-0.14	0.24

Spearman test, \* $p < 0.05$  strong correlation.

**TABLE 8.** Correlation analysis of degree of periodontitis and total cigarette consumption

Smokers	Severity of periodontitis (n/%)			<i>p</i>
	Mild	Moderate	Total	
Total cigarettes per day				
Light smokers	10 (41.7)	14 (58.3)	24 (31.6)	0.016*
Moderate smokers	30 (76.9)	9 (23.1)	39 (51.3)	
Heavy smokers	9 (69.2)	4 (30.8)	13 (17.1)	

Pearson  $\chi^2$  test, \* $p < 0.05$  strong correlation.

**TABLE 9.** Correlation analysis of IL-18 concentration and smoking duration

Smokers	Severity of periodontitis	
	<i>r</i>	<i>p</i>
Smoking duration	0.15	0.19

Spearman test, \* $p < 0.05$  strong correlation.

gression. IL-18, a proinflammatory cytokine in the IL-1 superfamily, induces IFN- $\gamma$  production in T cells and promotes the upregulation of Th1 and Th2 cytokines [11].

Our study demonstrated that the IL-18 concentration in gingival crevicular fluid increased in proportion with periodontal disease progression. IL-18 levels in smokers with moderate periodontitis were higher than in smokers with mild periodontitis. This increased IL-18 concentration was in agreement with previous reports. However, the results showed that the difference in IL-18 levels was not significant based on daily cigarette consumption, and there was no significant correlation with IL-18 concentrations in smokers based on the smoking duration using the Spearman test.

Pradeep *et al.* reported that IL-18 levels in gingival crevicular fluid increased with periodontal disease severity. In addition, IL-18 levels in the chronic periodontitis group decreased significantly after periodontal therapy [12]. Similar results were seen in the previous study by Nair *et al.*, which indicated that the IL-18 concentration in gingival crevicular fluid and serum was the lowest in subjects with good periodontal health, higher in gingivitis, even higher in aggressive periodontitis, and the highest in subjects with chronic periodontitis. They also reported that IL-18 concentrations in the gingival crevicular fluid and serum decreased 6–8 weeks after periodontal therapy [13].

These results may suggest that the preclinical alterations in periodontitis are triggered (and can be assessed) before the clinical alterations, as already mentioned. Determining the IL-18 concentration is useful in establishing an early diagnosis of alterations in the marginal periodontium of smokers. Moreover, IL-18 can be considered a potent inflammatory biomarker of periodontal disease.

Contrary to the results of this study, there was no significant difference between IL-18 levels in saliva samples of patients with chronic periodontitis and healthy subjects [11]. Similarly, there was no significant difference between IL-18 levels in gingival crevicular fluid in patients with chronic periodontitis and healthy subjects. When comparing the IL-18 level in saliva with that in gingival crevicular fluid, no significant difference was observed in either healthy subjects or patients with chronic periodontitis [14].

The correlation test results showed a significant relationship between periodontitis severity and the number of cigarettes consumed per day, but no significant correlation between periodontitis severity in smokers and smoking duration. Further studies with a similar methodology and investigative focus are needed to determine the precise relationship between smoking and gingival crevicular fluid profile in patients with chronic periodontitis.

## CONCLUSIONS

Based on the results of this study, the IL-18 concentration in gingival crevicular fluid increased in proportion with periodontal disease progression, and a significant relationship was found between periodontitis severity and the number of cigarettes consumed per day. However, differences in IL-18 levels were not significant based on daily cigarette consumption, and no significant correlation was found in IL-18 concentrations in smokers based on smoking duration. Consequently, IL-18 levels in saliva and gingival crevicular fluid can be used as a predictable biomarker for periodontal disease progression, but cannot be used to determine patients' smoking habits. Limitations of this study are that the re-

searchers did not categorize the types of cigarettes and the limitations due to the narrow age range of the subjects. Further studies involving larger sample sizes are warranted to confirm the role of IL-18 in the degree of chronic periodontitis in smokers.

## ACKNOWLEDGEMENTS

The study was self-funded by the authors and their institution.

## CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## References

1. Singh GH, Bhardwaj A, Singh Y. Smoking and periodontal disease. *J Pharm Sci Innov* 2013; 2: 7-13.
2. Mehta H, Nazzal K, Sadikot RT. Cigarette smoking and innate immunity. *Inflamm Res* 2008; 57: 497-503.
3. Abu-Ta'a M. The effects of smoking on periodontal disease: an evidence-based comprehensive literature review. *Open J Stomatol* 2014; 4: 33-41.
4. Marina T, Ioanna T, Maria M, Lazaros T. The effect of smoking on components of gingival crevicular fluid in patients with periodontal disease. *Oral Health Dent Manag* 2015; 14: 1-5.
5. Toma V, Cioloca DP, Forna DA, et al. IL 18 as an important gingival inflammatory biochemical marker in children and adolescents with insulin-dependent diabetes mellitus. *Rev Chim (Bucharest)* 2016; 67: 2545-2551.
6. Mokeem SA, Vellappally S, Preethanath RS, et al. Influence of smoking on clinical parameters and gingival crevicular fluid volume in patients with chronic periodontitis materials and methods after careful removal of all supragingival plaque, teeth were washed with water spray, isolated with cotton rol. *Oral Health Dent Manag* 2014; 13: 469-473.
7. Cetinkaya B, Guzeldemir E, Oguş E, Bulut S. Cytokines in gingival crevicular fluid and serum of patients with rheumatoid. *J Periodontol* 2012; 84: 84-93.
8. Kulczyk T. Evaluation of the results of periodontal treatment by means of digital subtraction of radiographic images. *Adv Med Sci* 2006; 51: 126-129.
9. Jain H, Mulay S. Detrimental effects of smoking on periodontium in health and disease. *Int J Sci Study* 2014; 2: 76-81.
10. Tagliacozzo R. Smokers self-categorization and the reduction of cognitive dissonance. *Addict Behav* 1979; 4: 393-399.
11. Eshahood ZR, Zare D, Reza JZ. Levels of interleukin-18 in saliva and gingival crevicular fluid in patients with chronic periodontitis and healthy subjects. *Annu Res Rev Biol* 2016; 10: 1-6.
12. 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: 261-266.
13. Nair V, Bandyopadhyay P, Kundu D, Das S. Estimation of interleukin-18 in the gingival crevicular fluid and serum of Bengali population with periodontal health and disease. *Indian Soc Periodontol* 2016; 20: 260-264.
14. Tanaka K, Miyake Y, Hanioka T, Furukawa S. The IL18 promoter polymorphism, rs1946518, is associated with the risk of periodontitis in Japanese women: The Kyushu Okinawa Maternal and Child Health Study. *Tohoku J Exp Med* 2017; 243: 159-164.