Analysis of Interleukin-1α Level in the Severity of Chronic Periodontitis Influenced by Smoking Habit

Adrianus Wicaksono[1], Sri Lelyati C Masulili[1*], Benso Sulijaya[1], Yulianti Kemal[1], Elza Ibrahim Auerkari[2]

1.Department of Periodontics, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia
2.Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia

Abstract

The accumulation of plaque that triggers the inflammatory and immune responses to bacterial plaque is a major cause of gingivitis and periodontitis. Chronic periodontitis is an infectious disease that causes inflammation of tissues supporting the teeth. The role of interleukin-1α (IL-1α) in chronic periodontitis and in smokers is unclear. This study aimed to analyse the IL-1α level in two categories, namely in chronic periodontitis and also in the smoker. A cross-sectional study of 104 subjects with chronic periodontitis, aged 33–78 years old, was conducted in the Teaching Dental Hospital, Faculty of Dentistry, Universitas Indonesia. Clinical data (Oral Hygiene Index score, pocket depth and Clinical Attachment Level), smoking status and gingival crevicular fluid (GCF) samples were collected, and the IL-1α level was detected by the ELISA test. No significant differences in the IL-1α level were found between the periodontitis groups of smokers and non-smokers (p=.70). No significant differences were also found between the group of smokers with mild–moderate and the severe periodontitis group (p=.06). Conversely, significant differences were observed in the group of non-smokers with mild–moderate periodontitis and the severe periodontitis group (p=.02). A relationship exists between the IL-1α level and the severity of chronic periodontitis but not smoking status.

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Introduction

Periodontal health is a dynamic state in which the activity of pro-inflammatory cytokines or antimicrobial to control infection optimally is commensurate with the anti-inflammatory mechanism to prevent inflammation.[1] The initiation and the progression of periodontal disease depend on the presence of microorganism bacterial plaque.[2] Specific microorganisms in the subgingival microflora are determinant agents for periodontitis, and the host defensive mechanism factor in periodontal tissue also plays an important role against periodontal damage.[3,4] Several important risk factors affect the aetiology of periodontal disease, namely, systemic conditions, smoking and age.[4,5]

The inflammatory response evidently plays an important role in the pathogenesis of periodontal disease. Interlekuin-1 (IL-1) is composed of IL-1α and IL-1β. IL-1 is a cytokine involved in the mediation of acute inflammatory disease and chronic inflammation, and it is mainly produced mainly by the stimulation of monocytes, macrophages, keratinocytes, smooth muscle cells and endothelial cells.[6] IL-1 shows the main activators of early cytokine and is responsible for the induction of adhesion molecules on endothelial cells, thus facilitating the migration of leukocytes from blood vessels into tissues. IL-1 also triggers an enzyme to produce prostaglandin E2 (PGE2), which plays a role in bone resorption and is a key regulator of matrix metalloproteinase and its inhibitor.[7]

Corresponding author:
Sri Lelyati C. Masulili
Department of Periodontics
Faculty of Dentistry, Universitas Indonesia
E-mail: srilelyati@yahoo.com

In periodontal disease, IL-1 is one of the active stimulator of osteoclast activity. Some evidence implicates pro-inflammatory cytokines, IL-1, in the pathogenesis of periodontal disease. IL-1α is a multifunctional inflammatory cytokine that is localised in the mononuclear cells, osteoclasts, osteoblasts and fibroblasts. The increase in IL-1α production has been reported to occur during the infectious inflammatory process and autoimmune diseases. IL-1α has been found to have a high concentration in gingival crevicular fluid (GCF) patients with periodontitis, although the concentration varies among different subjects.[2]

IL-1α should be analysed because it is a pro-inflammatory cytokine that occurs during inflammation and has a significant role in the pathogenesis of periodontitis. IL-1α has a biological activity similar to IL-1β and is bound to the same receptor with the same affinity. Moreover, studies showed that IL-1α is in the same concentration as IL-1β in GCF.[8] Therefore, this research was conducted because of the limited current research on IL-1α. The selection of GCF sampling as a result of IL-1α was detected in the gingival sulcus area, which is exposed by periodontal lesions in smokers.[8]

Given that previous researchers found that the IL-1α level increases in chronic periodontitis and that smoking can inhibit neutrophil chemotaxis, this study aims to analyse the IL-1α level in the clinical state of the periodontal tissue in chronic periodontitis groups of smokers and non-smokers. Several studies have examined the IL-1α level and found that average IL-1α concentrations were extremely lower in smokers (1.59±1.13 pg/μg protein) than in non-smokers (3.29±2.02 pg/μg protein).[8] Tymkiw et al. compared smokers with healthy control subjects and found that GCF in subjects with chronic periodontitis significantly contained a high number of the pro-inflammatory cytokine IL-1α.

The number of pro-inflammatory cytokines (IL-1α) decreased among smokers.[9] Neto examined the levels of IL-1α., -1ra, -6, -8 and RANKL and found that they were higher in smokers with periodontitis than in the control (group with healthy periodontal tissues) and that the levels of IL-10 and MMP-8 were lower (p<.00).[10]

The purposes of this research are to analyse the level of IL-1α in smokers with periodontitis severity, the differences in the level of IL-1α between smokers and non-smokers, and the differences in the IL-1α level between severe chronic periodontitis and mild chronic periodontitis.

Materials and method

The work was a cross-sectional research. Ethical clearance was given by the Ethical Committee, Faculty of Dentistry, Universitas Indonesia. The inclusion criteria for this study were chronic periodontitis and age >30 years old. The exclusion criteria were gingivitis, aggressive periodontitis, under medication within six months, systemic disease and patients undergoing an active periodontal treatment within three months. One hundred four male subjects were included in this study. GCF was taken by using a paper point and put inside Eppendorf tubes and then stored at −80 °C. The sample was tested using the enzyme-linked immunosorbent assay (ELISA kit IL-1α, KOMA Biotech, Korea). The concentration of IL-1α was examined using ELISA assay kits, and the result was analysed by a microplate reader.

The numerical descriptive data were inputted into the computer and analysed using SPSS 20.0. Data processing began with the descriptive statistical analysis and hypothesis testing. Descriptive statistics were used to present the data in the form of a frequency distribution of data centre size, such as the average (the value of mean), and in the form of a data spread size, such as standard deviation (SD). Statistics were tested by ANOVA and post-hoc with T-test. Data that considerably deviated from the normal distribution were analysed using the Mann–Whitney U test and the Kruskal–Wallis test to evaluate the statistically significant differences between the two groups. A p-value less than .05 was considered statistically significant.

Results

Data collection was conducted by clinical examination, questionnaires on smoking status and gingival sulcus fluid sampling. The selection of samples to be tested was conducted by consecutive sampling; 76 samples were grouped by chronic periodontitis (38 mild–

moderate samples and 38 severe samples) and smoking status (38 smokers and 38 non-smokers). The samples with mild–moderate chronic periodontitis were grouped into one because of the limited number of samples in the group.

Table 1 Normality Test on the IL-1α Level, Degree of Chronic Periodontitis Severity and Smoking Status.

Chronic Periodontitis	*p*-value
Mild–Moderate, Smokers	.00
Mild–Moderate, Non-smokers	.36
Severe, Smokers	.00
Severe, Non-smokers	.00

Shapiro–Wilk test; *p*>.05 normal data distribution.

The normality test of the clinical parameter on chronic periodontitis was conducted using the Shapiro–Wilk Test. The normality test showed that the distribution between the degree of chronic periodontitis severity and smoking status was abnormal; only the analysis between mild–moderate periodontitis and non-smokers was normal (*p*>.05).

Table 2 shows a comparative analysis between IL-1α level and degree of chronic periodontitis severity. A statistically significant difference (*p*=.01) was found between IL-1α level and the degree of chronic periodontitis severity. The IL-1α level of chronic periodontitis severity was higher than that of mild–moderate chronic periodontitis.

Table 3 shows the comparative analysis between IL-1α level of chronic periodontitis and smoking status. As indicated in Table 3, the average value of the IL-1α group of smokers with chronic periodontitis samples was lower (139.74 ±118.64 pg/mL) than that of non-smokers with chronic periodontitis (156.17 ±206.52 pg/mL). Statistically, no significant difference was found between the two groups (*p*=.70).

Table 2 Association between IL-1α Level and Degree of Chronic Periodontitis Severity.

Chronic Periodontitis	IL-1α Level (pg/mL)				
	N	Mean (SD)	Min-Max	CI 95%	*p-value*
Severity					.01
Mild–Moderate	38	104.11 (86.21)	3.95-456.62	75.77-132.44	
Severe	38	191.81 (213.27)	5.10-1075.99	121.71-261.91	

Mann–Whitney test; *p*<.05 significant difference

Table 3 Association between IL-1α Level of Chronic Periodontitis and Smoking Status.

Chronic Periodontitis	IL-1α Level (pg/mL)				*p-value*
	N	Mean (SD)	Min –Max	CI 95%	
Smoker					.70
Yes	38	139.74 (118.64)	3.49–468.73	100.75–178.74	
No	38	156.17 (206.52)	5.10–1.075.987	88.29–224.05	

Mann–Whitney test; *p*<.05 significant difference

The homogeneity test of variance with Levene's statistic experimental test was used as the second assumption in the average comparative test. When the value of the significance testing is greater than α = 5%, then the variation of the data in the sample group is homogeneous. The result of the SPSS v.20 processing is as follows:

Table 4 Analysis of the IL-1α Level between the Mild–Moderate and Severe Chronic Periodontitis Groups among Smokers

Smokers	N	IL-1α Level (pg/mL)		*p-value*
		Mean (SD)	Min-Max	
Severity of chronic periodontitis				.06
Mild–Moderate	19	109.17 (110.44)	3.95-456.62	
Severe	19	170.32 (121.50)	69.17-468.73	

Kruskal–Wallis Test; $p<.05$ significant difference

The above tables show a meaningful test value of .06 >.05, which indicates that H_0 is acceptable. Therefore, the average IL-1α level in the group of mild–moderate chronic periodontitis samples did not differ significantly from that of the group with severe chronic periodontitis samples among smokers.

Discussion

This study shows that IL-1α may be a good indicator of inflammation. However, the results according to the Kruskal–Wallis and Mann–Whitney tests reject the major hypothesis (i.e. the lack of significant differences in the IL-1α level between the severe chronic periodontitis and mild–moderate chronic periodontitis groups in smokers) and the first minor hypothesis (i.e. the IL-1α level has no significant difference between the smoker and the non-smoker groups). When the smokers and non-smokers are combined, the results of the study show a higher level of IL-1α in the severe chronic periodontitis group than in the mild–moderate chronic periodontitis group. These results are consistent with those of Mathur et al., which show that the IL-1α level in severe chronic periodontitis is higher than in mild–moderate chronic periodontitis.

Ojima et al. found a decrease in the IL-1α level in smokers but not in non-smokers.[11] Petropoulos et al. showed that the average of the IL-1α level in smokers was less than half of that in non-smokers (smokers 1.59±0.3 pg/mg protein; non-smokers 3.29±0.46 pg/mg protein, p=.01, Mann–Whitney test).[8] Furthermore, in Tymkiw et al., GCF in the chronic periodontitis group (both smokers and non-smokers) contained a significantly higher number of IL-1αl level than in the healthy control subjects, whereas the IL-1α level among smokers with chronic periodontitis decreased.[9]

This finding is consistent with that of Cesar-Neto et al.; that is, the IL-1α level was higher in smokers with periodontitis than in the healthy subjects. Smoking also lowered the IL-1α level in the subjects with chronic periodontitis.[10]

One possible reason is that pro-inflammatory mediators occur in severe periodontal circumstances, which cause periodontitis. The current study was conducted on subjects with chronic periodontitis, who were not compared with healthy subjects.

Periodontitis is a chronic inflammatory disease that attacks the structures supporting the teeth, and it is triggered by periodontal pathogen bacteria and clinical damage due to the local host response. Lipopolysaccharides, which are contained in the cells of Gram-negative bacteria, act as a powerful stimulant of host response. The increase in polymorphonuclear (PMN) cells in an area is followed by the release of cytokines by neutrophils and macrophages. Chemical mediators, such as tumour necrosis factor-α and IL-1α, are also released. The inflammatory process that occurs is in the form of fibroblast

stimulation by IL-1α and secretion of matrix metalloproteinase (MMP) by PMN. The stimulation of fibroblasts and macrophages may induce the production of prostaglandins. The presence of cytokines, prostaglandins and MMP in turn causes damage to the bone.[12]

This study can be extended by increasing the number of samples to compare the sampling retrieval on the same individual but not in different variations of CAL and by conducting the analysis on healthy individuals as a control group.

Conclusion

The IL-1α level in severe chronic periodontitis is higher than that in mild–moderate chronic periodontitis. Moreover, IL-1α is lower in smokers than in non-smokers. The IL-1α level in smokers with severe chronic periodontitis does not differ from that in smokers with mild–moderate chronic periodontitis.

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Declaration of Interest

The authors report no conflict of interest.

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