Analysis of the Relationship between Interleukin-12 and Chronic Periodontitis in Smokers and Non-Smokers

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Abstract
Chronic periodontitis is an inflammation of periodontium, result of the host response to bacterial accumulation on the tooth surface, and smoking can alter the host's response to plaque accumulation. Interleukin-12 plays a role in the regulation of the immune response of the host. We aimed to examine the relationship between IL-12 levels in gingival crevicular fluid and chronic periodontitis severity degree in smokers and non-smokers. 

A cross-sectional study comprising 76 male patients, aged 20-35 years old. Forty-six smokers, and 36 non-smokers. Sixty-four with periodontitis, and 12 healthy subjects. Patients clinically evaluated, and gingival crevicular fluid was retrieved using paper points from which IL-12 levels determined using an enzyme-linked immunosorbent assay. IL-12 levels were higher in patients with chronic periodontitis than in healthy individuals (p=0.042). IL-12 levels in the smoker group were higher than those in the non-smoker group (p=0.012). IL-12 levels were directly proportional to the increase in chronic periodontitis severity degree (p=0.001).

As a conclusion, there was a significant relationship between IL-12 levels and chronic periodontitis severity degree. IL-12 levels were higher in patients with chronic periodontitis than in healthy individuals and were also directly proportional to the increase in periodontitis severity degree. IL-12 levels in the smoker group were also higher than those in the non-smoker group.


Keywords: Chronic periodontitis, Interleukin-12, Gingival crevicular fluid, Smoking.

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Introduction
Periodontal disease can result in inflammation and damage from the attachment of the dental apparatus.¹ Periodontitis is the result of the host response to bacterial accumulation on the tooth surface, and bad habits, such as smoking. Can lead to the acceleration of disease progression by altering the host’s response to plaque accumulation.² Several studies examining cytokine levels in the gingival crevicular fluid have found that an imbalance between pro-inflammatory and anti-inflammatory mediators may cause periodontal disease. IL-12 is a pro-inflammatory cytokine that plays a role in the regulation of the immune response of the host.³ Periodontitis is the second most common dental and oral disease in Indonesia. National Household Health and Health Survey data shows that 42.8% of Indonesia's population suffers from this disease.⁴ The accumulation of plaque and calculus in the gingival sulcus region is one of the common symptoms of periodontal disease and leads to an inflammatory response, the later stages of which can trigger an immune response that can lead to tissue damage.⁵ Periodontal disease defined as a condition that can cause inflammation and suffering from the attachment of the dental apparatus.¹ It is initiated by an oral bacterial invasion that induces localized inflammatory responses, bleeding on probing, and loss of periodontal attachment between the bones and teeth.⁶

The microorganisms that cause periodontitis also cause gradual damage to the periodontal ligament and alveolar bone, resulting in pocket formation, recession, or both. The American Academy of Periodontology (AAP,
1999) classifies periodontitis into three groups: chronic periodontitis, aggressive periodontitis, and periodontitis as a manifestation of systemic disease.

Chronic periodontitis involves inflammation of the periodontal tissues caused by microorganisms in the plaque, i.e., Gram-negative anaerobic bacteria such as Porphyromonas gingivalis, Tannerella forsythia, Prevotella intermedia, and Aggregatibacter actinomycetemcomitans. The inflammation is due to a host response to bacterial aggregation on the tooth surface, and habits, such as smoking, can lead to the accelerated progression of disease because they may alter the host's response to plaque accumulation. It is known that smoking could change cell function and trigger the development and increase in disease severity degree. Smokers also exhibit an increase in alveolar bone damage and tooth loss compared with non-smokers. Increased severity of the disease depends on the intensity and duration of cigarette exposure.

Protective responses to periodontitis involve secretion of inflammatory and anti-inflammatory cytokines, which play essential roles in the immune system. Periodontal tissue damage will cause by a complex interaction between pathogenic bacteria and the host immune response. Several studies have examined cytokine levels in the gingival crevicular fluid (GCF), and an imbalance between pro-inflammatory and anti-inflammatory mediators is believed to cause periodontal disease. Interleukin-12 (IL-12), which is a pro-inflammatory cytokine, plays a role in the regulation of the immune response of the host and is naturally produced by dendritic cells, macrophages, and human B-lymphoblastoid cells (NC-37) in response to antigenic stimulation. IL-12 comprises a bundle of four alpha helices and is a heterodimeric cytokine encoded by two separate genes IL-12A (p35) and IL-12B (p40). It plays an essential role in activities of natural killer cells and T lymphocytes by mediating their cytotoxic activity. It also exerts anti-angiogenic activity. IL-12 p40 exists as a monomer and homodimer, and IL-12B contains eight exons and maps to 5q31-q33.

According to Sanchez et al., IL-12 levels in the gingival tissue significantly elevated in patients with periodontitis, and serum IL-12 levels are also considerably higher in patients with chronic periodontitis compared with controls (healthy individuals). These findings suggest the involvement of a classical mechanism of immunopathogenesis in this form of periodontitis.

In this study, we have compared the smoker group to explore the correlation of IL-12 with various degree of periodontitis in the male population.

## Materials and methods

This method was a cross-sectional study which received ethical approval from The Ethical Committee of Dental Research (KEPKG) 2018 and conducted at the Department of Periodontics and Oral Biology, Kelapa Dua Depok, Indonesia. The study conducted between March and April 2018 on 76 male subjects. Patients were asked to complete questionnaires, and laboratory tests to analyze IL-12 levels in the sampled GCF were performed at the Integrated Laboratory of Faculty of Dentistry, Universitas Indonesia, using enzyme-linked immunosorbent assay (ELISA). Statistical analysis was performed using SPSS software.

The inclusion criteria were a diagnosis of chronic periodontitis, history of smoking, willingness to participate in the study, and provision of informed consent. The exclusion criteria were mental retardation, history of aggressive or systemic periodontal disease, or periodontal treatment within the previous three months.

Chronic periodontitis defined as a periodontium health condition (clinical parameter) assessed through clinical attachment loss (CAL). The classification of periodontitis severity degree in this study was as described by Page and Eke (2007), which divides chronic periodontitis into severe, moderate, and mild forms. Severe periodontitis is defined as CAL ≥ 5 mm, moderate as CAL of 3–4 mm, and mild periodontitis as CAL = 0–2 mm.

Patients who meet the inclusion criteria provided informed consent and anamnesis; a single periodontal consultant examined plaque index (PII), PBI, PD, and LoA. The GCF sample collected in three steps. First, the supragingival plaque was removed using curettes and the tooth surface was dried with a cotton roll. Second, three pieces of a no.10 paper point were inserted
into the pocket and left for 30 s. Third, the paper points were removed and placed into a sterile tube containing 200 µl Tris-EDTA (TE) buffer. The samples were placed in a cool box and immediately taken to the laboratory and frozen at −80°C until required. For ELISA anti-IL-12 (P40) polyclonal antibody was pre-coated onto 96-well plates for use as a capture antibody. Standards, test samples, and biotin-conjugated detection antibody (biotin-conjugated P40 polyclonal antibody) and were subsequently added to the wells which then washed with the wash buffer. Avidin-biotin-horse peroxidase (HRP) was added and unbound conjugates were washed away with the wash buffer. TMB was used for visualizing the HRP enzymatic reaction, which was read as the absorbance at 450 nm using a microplate reader. The IL-12 (P40) level was calculated based on a standard curve, and data analysis was performed using SPSS software.

Results

Subjects divided into two groups: smoking and non-smoking. The total subject were 76 males and 36 subjects were classified into the non-smoking group and 40 subjects belonged to the smoking group. Data presented in Figure 1.

![Figure 1. Subject Distribution based on their Smoking Status.](image)

Table 1 shows the distribution of the subjects based on their periodontitis status and severity degree. A total of 64 (84.21%) subjects suffered from periodontitis and the remaining 12 (15.79%) were healthy. Subjects with periodontitis were most commonly found to have a moderate degree of disease (34 subjects; 53.12%). Severe periodontitis observed in 26 (40.63%), and mild periodontitis observed in 4 (6.25%) subjects.

<table>
<thead>
<tr>
<th>Subjects Distribution</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Status of Periodontitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>12</td>
<td>15.79</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>64</td>
<td>84.21</td>
</tr>
<tr>
<td>Degree of Periodontitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>4</td>
<td>6.25</td>
</tr>
<tr>
<td>Moderate</td>
<td>34</td>
<td>53.12</td>
</tr>
<tr>
<td>Severe</td>
<td>26</td>
<td>40.63</td>
</tr>
</tbody>
</table>

Table 1. Subject Characteristics.

IL-12 levels determined in this study are presented in Table 2. The mean IL-12 level in subjects who smoked was higher (9.77 ± 4.64 pg/mL) than that in subjects who did not smoke (6.73 ± 1.14 pg/mL). Subjects with periodontitis had higher IL-12 levels than healthy subjects (8.70 ± 3.98 and 6.33 ± 0.67 pg/mL, respectively). IL-12 levels in subjects with severe periodontitis were the highest (11.12 ± 4.99 pg/mL), followed by the those in subjects with moderate (6.76 ± 1.45 pg/mL) and mild (8.77 ± 1.90 pg/mL) periodontitis.

<table>
<thead>
<tr>
<th>Subject</th>
<th>N</th>
<th>IL-12 Levels (pg/ml)</th>
<th>Mean (SD)</th>
<th>Min - Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smokers</td>
<td>36</td>
<td>6.73 (1.14)</td>
<td></td>
<td>5.33 – 9.98</td>
</tr>
<tr>
<td>Smokers</td>
<td>40</td>
<td>9.77 (4.64)</td>
<td></td>
<td>5.33 – 19.04</td>
</tr>
<tr>
<td>Status of Periodontitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>12</td>
<td>6.33 (0.67)</td>
<td></td>
<td>5.33 – 7.72</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>64</td>
<td>8.70 (3.98)</td>
<td></td>
<td>5.33 – 19.04</td>
</tr>
<tr>
<td>Degree of Periodontitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>4</td>
<td>8.77 (1.90)</td>
<td></td>
<td>5.94 – 9.98</td>
</tr>
<tr>
<td>Moderate</td>
<td>34</td>
<td>6.76 (1.45)</td>
<td></td>
<td>5.33 – 13.79</td>
</tr>
<tr>
<td>Severe</td>
<td>26</td>
<td>11.12 (4.99)</td>
<td></td>
<td>5.33 – 19.04</td>
</tr>
</tbody>
</table>

Table 2. Distribution of Average IL-12 Levels Based on the Smoking Status, Periodontitis Status, and Periodontitis Degrees.

A data normality test was performed to determine the distribution of IL-12 levels concerning the smoking status, periodontitis status, and periodontitis severity degree using the Shapiro–Wilk test, except for healthy periodontitis status with samples over 50, where the Kolmogorov–Smirnov test used. All variables gave values of p < 0.05; therefore, data were assessed using nonparametric tests.
Periodontitis, defined as an inflammatory disease of supportive tissue of teeth caused by the specific microorganism which lead to progressive destruction of periodontal membrane and alveolar bone, with the formation of periodontal pockets and gingival recession. The incidence and progression rate of periodontal disease depends on the complex interaction between periodontopathic bacteria, cells of the host immune system and environmental factors. Smoking is such a factor that can place a host at higher risk of developing periodontal disease. Smoking can alter cell function and trigger an increase in alveolar bone damage and tooth loss compared with not smoking; also, increased disease severity depends on the intensity and duration of cigarette exposure.

The interaction between bacterial agents, the environment, and the host defense mechanism's response to bacterial attacks affect periodontitis severity. Periodontal tissue abnormalities may occur or persist if homeostasis between pro-inflammatory mediators and anti-inflammatory mediators achieved. The presence of etiopathogenic bacteria that initiate inflammation leads to a change in the balance between pro-inflammatory and anti-inflammatory cytokines, which play a significant role in periodontitis pathogenesis. In our findings, IL-12 elevation was correlated with periodontitis severity.

Some study has reported the same results with our findings. Tsai et al. have found the total amount of IL-12 was significantly higher in chronic periodontitis sites than gingivitis or healthy sites of non-smokers/non-alcohol drinkers. It means that IL-12 plays a potential role in the destruction of periodontitis.

On the contrary, Moeintaghavi et al. found that IL-1β gene expression has increased in gingival tissue of non-smoker-chronic periodontitis patients due to inflammatory processes, but smoking has reduced the appearance of this cytokine in diseased
periodontal tissues. They also found that periodontal condition and smoking habits do not seem to affect IL-12 gene expressions in gingival tissues. Another study also indicated that the level of IL-12 decreased in the test group compared with the control group, but there was no significant difference between the two groups. Very little IL-12 has been detected with levels decreasing with increased inflammation in GCF samples of periodontitis patients. Considering the available data, it seems that the main difference in the studies is related to sample collection, test protocols, and genetically different populations. Thus, future research focusing on local tissue sample or GCF using more advanced methods is needed to establish these findings.

Conclusions

There was a relationship between IL-12 levels and chronic periodontitis severity degree. Levels of IL-12 were higher in subjects with chronic periodontitis than in healthy subjects and were also directly proportional to the increase in chronic periodontitis severity. IL-12 levels in the smoker group were higher than those in the non-smoker group. This study is useful as a novel study that compares IL-12 levels against other periodontal diseases, such as aggressive periodontitis.

Declaration of Interest

The authors declare that there are no conflicts of interest and want to acknowledge the support of Integrated Laboratory of Faculty of Dentistry, Universitas Indonesia, and also PITTA grant from Universitas Indonesia, Jakarta.

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