**Association of interleukin 8 -251 A/T gene polymorphism with periodontitis in Indonesia**

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**Abstract.** Periodontitis is a chronic multifactorial disease resulting from an interaction between periodontal pathogen bacteria, host, and environmental factors. Genetics has been identified to contribute to the pathogenesis and susceptibility of periodontitis, and interleukin 8 (IL-8) gene is expressing one of the chemokines involved in the inflammation process. This study aimed to evaluate the association of IL-8 -251 A/T gene polymorphism with periodontitis in Indonesian subjects. The study was conducted by genotyping 72 samples of patients with various severity of periodontitis and 41 samples of healthy controls group using PCR-RFLP method. Genotypes of the IL-8 gene polymorphism in periodontitis patients were not significantly different with those in healthy controls. Those with TT genotype were 3.40 times less likely to develop periodontitis compared to AA genotype (95% CI). There was a significant difference in allele frequencies (p < 0.05, OR = 1.828, 95% CI), suggesting that allele T is a risk factor to periodontitis. Statistical analyses with Chi-square testing showed no significant association of IL-8 -251 A/T gene polymorphism and the severity of periodontitis in Indonesia. However, IL-8 -251 A/T gene polymorphism might contribute to the susceptibility of periodontitis.

**1. Introduction**

Periodontitis is a chronic inflammation in periodontium, and globally most common oral disease after caries. As a widespread burden to oral health, prevalence of periodontitis is still considered to be very high in many countries [1, 2]. Around 10 – 15% of the population worldwide, aged 35 to 44 years, has severe periodontitis [3]. In developing countries like in Indonesia, prevalence of periodontitis can be more than 70% [4].

Periodontitis is characterized by loss of attachment, periodontal pocket formation, and reduction in height and density of alveolar bone. These processes may lead to tooth loss [5]. Consequently, periodontitis patients can face difficulties in functioning.

Periodontitis results from an interaction between periodontal pathogen bacteria, host, and environmental factors. As a complex multifactorial disease, pathogenesis of periodontitis is determined by a number of contributing factors, including genetic ones. It is acknowledged that polymorphisms of the host genes can be closely associated with the susceptibility to periodontitis [6].

Among the chemokines that might contribute in the pathogenesis of periodontitis, interleukin 8 (IL-8) has been identified as one that is involved in the inflammation process. IL-8 is a chemotactic factor for neutrophils produced by various types of cells such as macrophages, epithelial cells, and endothelial cells. Elevated IL-8 levels have been reported in inflamed periodontal tissue and gingival crevicular fluid in patients with periodontitis [7].
Considering the important role of IL-8 in pathogenesis of periodontitis, this study aims to evaluate the potential association of IL-8 $-251$ A/T gene polymorphism with severity of periodontitis in Indonesian subjects.

2. Materials and methods

2.1. Study population
This study was performed with the written approval of the Ethics Committee of the Faculty of Dentistry, University of Indonesia. The case-control population consisted of 113 subjects. The patient group consisted of 72 male adults, aged 25 to 60 years. Subjects were selected from patients of Central Hospital of Cipto Mangunkusumo, Jakarta. The blood samples from each subject were collected and purified to get the DNA extracts by the methods described by Auerkari et al. The DNA samples were stored in $-20^\circ \text{C}$ – $-80^\circ \text{C}$ before use [8, 9]. The patient group had been classified for the severity of periodontitis according to the 1999 International Workshop for a Classification of Periodontal Diseases and Conditions (mild periodontitis = 1 – 2 mm, moderate periodontitis = 3 – 4 mm, and severe periodontitis = $\geq$ 5 mm) [10]. The control group consisted of 41 healthy subjects (16 males and 25 females, aged 17 to 58 years) who visited the university as blood donors.

2.2. Genotype analysis
The polymorphism was analyzed with polymerase chain reaction (PCR) amplification technique, followed by restriction fragment length polymorphism (RFLP). The promoter region was determined using specific forward primer (5’-CCA TCA TGA TAG CAT CTG TA-3’) and reverse primer (5’-CCA CAA TTT GGT GAA TTA TTA A-3’) [11]. PCR reactions were performed using conventional PCR machine in a total volume of 18 µL containing 10 µL master mix (Bioline), 0.5 µL of each primer, 6.5 µL ddH$_2$O, and 0.5 µL genomic DNA. PCR was performed at 94$^\circ \text{C}$ for 5 min, followed by 35 cycles of 30 s at 94$^\circ \text{C}$; 30 s at 54$^\circ \text{C}$; 1 min at 72$^\circ \text{C}$; and a final elongation at 72$^\circ \text{C}$ for 8 min. The 173 bp product was digested with VspI (Thermo Fisher Scientific) for 16 hours at 37$^\circ \text{C}$. The digested PCR products generate two fragments (152 bp and 21 bp) for allele A (wild-type) and 173 bp for allele T (mutant-type). The fragments were separated by electrophoresis in 3% agarose gel stained with GelRed for visualization using Gel Doc.

![Figure 1. PCR-RFLP assay for genotyping the IL-8 $-251$ A/T polymorphism.](image)

2.3. Statistical analysis
Hardy-Weinberg equilibrium analyses with Chi-square testing were used for evaluating the distribution of IL-8 $-251$ A/T gene polymorphism. The frequencies of severity of periodontitis, genotypes, and alleles were compared using Chi-square test. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated to assess the associated relative risk. Statistical analyses were performed by SPSS 17.0 software (SPSS Inc., Chicago, IL, USA).
3. Results
3.1. Characteristics of the study population
The characteristics of case-control study population are presented in Table 1. All 72 male periodontitis patients had a mean (±SD) age of 39.07 (± 9.58) years. The control group of 41 subjects consisting of 16 males and 25 females had a mean (±SD) age 24.32 (± 10.09) years. There were more smokers in the patient group than in the control group.

| Table 1. Demographic characteristics of patient and control groups |
|---------------------------------------------------------------|
| Clinical Form | Mild Periodontitis | Moderate Periodontitis | Severe Periodontitis | Healthy Control |
| Frequency | 8 males | 37 males | 27 males | 16 males, 25 females |
| Age range (years) | 26 – 51 | 25 – 54 | 25 – 60 | 17 – 58 |
| Mean age ± S.D. (years) | 37.50 ± 9.971 | 36.65 ± 8.728 | 42.85 ± 9.706 | 24.32 ± 10.091 |
| Smoking status | - Nonsmoker | 5 | 17 | 8 |
| | - Smoker | 3 | 20 | 19 |

3.2. Genotype and allele distributions in periodontitis patients and controls
The distributions of genotypes both in periodontitis and control subjects were found to be in agreement with the Hardy-Weinberg equilibrium (χ² < 3.841, p = 0.059 for periodontitis group and χ² < 3.841, p > 0.05 for control group). The genotype and allele frequencies of IL-8 in periodontitis patients and controls are presented in Table 2. Genotypes of the IL-8 gene polymorphism in periodontitis patients were not significantly different with those in healthy controls (p > 0.05). However, compared to subjects with AA genotypes, those with TT genotype were 3.40 times less likely (95% CI = 1.067 – 10.836) to have periodontitis. Conversely, there was a significant difference in the allele frequencies (p < 0.05, OR = 1.828, 95% CI = 1.046 – 3.194), suggesting that allele A is a risk factor for periodontitis.

| Table 2. Genotype and allele frequencies of IL-8 –251 A/T polymorphism in periodontitis patients and controls |
|---------------------------------------------------------------|
| IL-8 -251 A/T | Periodontitis n = 72 | Control n = 41 | p | OR | 95% CI |
| AA | 22 (30.6%) | 5 (12.2%) | 4.935 | 0.085 |
| AT | 28 (38.9%) | 19 (46.3%) | 2.986 | 0.962 – 9.265 |
| TT | 22 (30.6%) | 17 (41.5%) | 3.400 | 1.067 – 10.836 |
| Allele A | 72 (50%) | 29 (35.4%) | 4.527 | 0.033 |
| Allele T | 72 (50%) | 53 (64.6%) | 1.828 | 1.046 – 3.194 |

3.3. Genotype and allele frequencies of IL-8 –251 A/T polymorphism and periodontitis
There was no significant association between IL-8 –251 A/T polymorphism and severity of periodontitis. As presented in Table 3, there were no significant differences between genotypes and allele frequencies of IL-8 –251 A/T polymorphism in relation to severity of periodontitis (p > 0.05).
Table 3. Genotype and allele frequencies of IL-8 –251 A/T polymorphism in relation to severity of periodontitis

| IL-8 –251 A/T | Periodontitis | p  | OR   | 95% CI |
|--------------|--------------|----|------|--------|
|              | Mild and Moderate | n = 45 |    |        |
| AA           | 15 (33.3%)  | 7 (25.9%) | 0.658 | 0.720  |
| AT           | 16 (35.6%)  | 12 (44.4%) | 1.607 | 0.500 – 5.170 |
| TT           | 14 (31.1%)  | 8 (29.6%) | 1.224 | 0.351 – 4.269 |
| Allele A     | 46 (51.1%)  | 26 (48.2%) | 0.119 | 0.731  |
| Allele T     | 44 (48.9%)  | 28 (51.8%) | 1.126 | 0.573 – 2.211 |

4. Discussion
Risk of periodontitis is determined by multiple factors, and genetics has a role as risk factor to periodontitis [12]. Among the genes that might contribute to susceptibility of periodontitis, IL-8 is thought to be one of the mediators involved in inflammatory process. The IL-8 gene resides in chromosomal location 4q13-q21. The gene consists of four exons, three introns, and a proximal promoter region. The –251 A/T functional polymorphism is located in the promoter region, and has been reported influence the production and expression of IL-8 [13, 14].

Also, previous studies suggest variation in the frequency of the polymorphisms between populations [15 – 17]. Our results showed no significant association of IL-8 –251 A/T gene polymorphism with the severity of periodontitis in Indonesian subjects. However, this polymorphism might contribute to the susceptibility of periodontitis.

In our study, the frequency of A allele among the healthy controls was 35.4%. This is similar to the frequency observed in healthy controls in Iran and Brazil (35.2% and 36%, respectively) [18, 19], but lower than in Chinese population (47.6%) [20]. Moreover, there was significant difference in allele frequencies between the periodontitis and the healthy control group (p < 0.05, OR = 1.828, 95% CI = 1.046 – 3.194). This suggests that allele T is not a risk factor of periodontitis. Some previous studies have also reported such associations in Chinese, Brazilian, and Iranian populations [16-18]. However, the T allele frequency was found to be higher in periodontitis group than in healthy controls for the Chinese population [18]. In contrast, the A allele frequency was higher in periodontitis groups than in the controls groups in the studies of Brazilian and Iranian populations [18, 19]. The results of our study were in general agreement with the latter studies, except for similar A and T allele frequencies in the periodontitis patients group of the present work.

To the best of our knowledge, the present study is the first one that aims to evaluate the association of genotype and allele distributions of IL-8 gene polymorphism with the severity of periodontitis. However, we found no significant association in both distributions of IL-8 gene polymorphism (IL-8 –251 A/T) with the severity of periodontitis (p > 0.05). The results showed that if such an association can be shown by e.g. larger size of sampling, it must be relatively weak in comparison with other known contributing factors to the severity of periodontitis, such as virulence of bacteria, other gene polymorphisms, systemic diseases, and oral habits [21]. Further studies are suggested for clarification concerning limitations of the sample size, and non-matched sampling that could introduce confounding factors.

5. Conclusion
In conclusion, IL-8 –251 A/T gene polymorphism was found to show no association with the severity of periodontitis in Indonesian subjects. However, this polymorphism may contribute to the susceptibility of periodontitis.
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