



The Analysis relationship of Interleukin-1B (+3954) gene polymorphisms in Indonesian Population with Aggressive Periodontitis and Chronic Periodontitis

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Abstract: This research is aimed to determine the relationship of IL-1B (+3954) gene polymorphisms in aggressive periodontitis and chronic periodontitis in Indonesian population since recent researches suggested that IL-1B (3954) gene polymorphisms may become a risk factor for aggressive periodontitis. This research was observational analytical research with case control study design among two groups of patients. The population of this research were aggressive and chronic periodontitis patients who came to the Periodontics Clinic, Faculty of Dentistry, Airlangga University, Surabaya, Indonesia. All subjects were Indonesian population. Forty of them had suffered from aggressive periodontitis, and 40 of the others had suffered from chronic periodontitis. Genomic DNA was then extracted from the whole-blood samples, and IL-1B (+3954) gene polymorphisms were analyzed by *polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)*. This research showed that group suffered from chronic periodontitis had a higher percentage of CT allele even though it was not statistically significant. It also showed that IL-1B (+3954) gene did not affect those patients with either aggressive or chronic periodontitis. Based on the result of IL-1B polymorphisms test, it could be concluded that there was no relation between IL-1B (+3954) gene polymorphisms in aggressive periodontitis and chronic periodontitis in Indonesian population.

Keywords : Periodontitis, Interleukin-1B genotype, polymorphisms, PCR-RFLP.

Introduction

Periodontal disease commonly known as periodontitis is an infectious disease caused by various factors, which can easily cause periodontal tissue damage. The etiopathogenesis of periodontitis is actually considered of the interaction between host and bacterial plaque, but often misdiagnosed so that the treatment yields in unsatisfactory results. However, there are many patients suffering from severe periodontal tissue destruction without the accumulation of bacterial plaque¹.

In fact of periodontitis, it is a complex genetic disease which phenotype is determined by the structure of genes. Some researches show that the variation of the host immune response is associated with genetic factors and plays an important role in the occurrence of aggressive periodontitis and chronic periodontitis^{2,3}. In

America many researches reported higher occurrence of aggressive periodontitis in African-Americans race than that in Caucasian indicating that they are vulnerable to it, about 10%^{2,4}.

In addition periodontitis is a polygenic disorder in which many genes affecting the disease. Polymorphisms occurs as the result of gene mutation, and all organisms will actually get spontaneous mutation as a result of normal cell function or interactions with environment. *Single nucleotide polymorphism* (SNP), moreover, is one of the variations that occurs only as the impact of change and considered as the most common type. SNPs even have important biological effects due to both of the changes of proteins structure that can alter the function, and the mutations of gene promoter. It can also alter gene regulation and determine good or bad condition of individuals while reacting to certain situations^{5,6}. Recent reports showed that genetic variation in cytokine genes and factors regulate their expression may influence the clinical outcome, susceptibility and progressivity of periodontal disease. The interleukin -1 family of cytokines has a wide range of activities involved in the genesis and maintenance of inflammatory responses. Interleukin-1 is a pro-inflammatory protein which major function as mediator and regulator of host inflammatory responses in innate immune system and plays a role in a number of biological activities including proliferation, development, homeostasis, regeneration, repairing, and inflammation^{7,8}.

Several studies showed the role for interleukin-1 gene cluster polymorphisms in the risk assessment for periodontal disease^{5,7,8}. There were actually three genes which regulated the production of IL-1, such as IL-1A, IL-1B, and IL-1 RN. IL-1B gene was associated with the production of IL-1 β associated with inflammation. *In vitro* researches, there were evidence about direct relation of IL-1 genotype and the amount of cytokine released in macrophage culture⁹. Thus, IL-1B polymorphism will be associated with the severity of periodontitis. This research is aimed to identify genetic factors in a bid assess, the vulnerability of patients towards aggressive periodontitis and chronic periodontitis¹⁰. Based on the above descriptions, therefore, it is necessary to identify the genetic variations of IL-1B (+3954) as a risk factor for aggressive periodontitis and chronic periodontitis. This research was also expected to reveal the basic pathogenesis of aggressive periodontitis and chronic periodontitis, as well as to be able to be used in determining the risk factors of aggressive periodontitis and chronic periodontitis. In the long term, it will also useful for protecting us from the disease, by detecting it in earlier.

Material and Method

This research was an observational analytic study with case-control study designed in patients suffering from aggressive periodontitis and chronic periodontitis. Gene variant test of IL-1B (+3954) was conducted with PCR-RFLP. The population of this research were all patients who came to the periodontic clinic, Faculty of Dentistry, Airlangga University. The number of cases consisted of 40 cases of aggressive periodontitis, and 40 cases of chronic periodontitis as control group. Samples of this research, however, involved only all of those patients suffering from aggressive periodontitis and chronic periodontitis in Clinical Periodontia who had already signed 'Statement of Approval' to be willing participate in this research and had met inclusion and exclusion criteria determined in this research.

The sample collection and preparation techniques conducted in this research, were as the following. First, samples of blood, approximately 3ml, were taken from all patients diagnosed with chronic periodontitis and aggressive periodontitis. Second, samples preparation were conducted for molecular analysis, DNA extraction, and DNA amplification conducted by using PCR about 32 cycles. Third, primers used had to be in accordance with specific primer sequences of IL-1B (+3954), F: 5'-CTC AGG TGT CCT CGA AGA AAT CAA A-3 'and R: 5'-GCT TTT TTG CTG TGA GTC CCG-3.' Fourth, the visualization of PCR IL-1B products was conducted by agarose gel 3% and by adding with ethidium bromide 1 μ l placed in gel electrophoresis apparatus. Fifth, electrophoresis was then conducted at 100V, 70 MAMP for 40 minutes followed documentation by using Gel Doc system. Sixth, the determination of IL-1B (+3954) polymorphism was conducted with PCR-RFLP techniques by using TaqI specific restriction enzyme.

In addition, the criteria for IL-1B (3954 +) polymorphism were: (a) allele 1 [C]: 85bp + 97 bp (normal homozygote), (b) allele 2 [T]: 182bp (mutant homozygote), (c) allele 1+2 [C + T] : 182bp and 85bp + 97bp (heterozygote). The restriction (incubation) was then conducted at 37 °C for overnight in an incubator. Next, the results of DNA (RFLP) fragments were detected by electrophoresis using a high resolution of agarose 3% mixed with Ethidium bromide 1 μ l. At the time of the application of DNA agarose was mixed with loading

buffer 2 µl and DNA fragments 15 µl run on electrophoresis equipment at 100 Volt for 40 minutes. The results were then read by using Gel Doc system, and recorded.

Results

The research data is displayed in the form of tables and photographs arranged based on the design of this research as the following:

Table 1. Cross Tabulation of IL-1B (+3954) Gene Polymorphism on Patients Suffering from Aggressive Periodontitis (AP) and Chronic Periodontitis (CP)

Gene IL-1B varian	AP	Percentage	CP	Percentage	TOTAL
Allele 1 [C]	19	47.5	14	35	33
Allele 2 [T]	3	7.5	1	2.5	4
Allele 1+2 [CT]	18	45	25	62.5	43

Table 1 showed IL-1B (+3954) gene polymorphism based on the respons of those aggressive periodontitis patients and those chronic periodontitis ones. The occurrence of allele 1 [C] in those aggressive periodontitis patients was about 47.5%, and that in those chronic periodontitis patients was about 35%. Meanwhile, the occurrence of allele 2 [T] in those aggressive periodontitis patients was about 7.5% (3 respondents), and that in those chronic periodontitis patients was only about 2.5% (1 respondent). On the other hand, the occurrence of alleles 1 +2 [CT] was much different, about 45% in those aggressive periodontitis patients, and 62.5% in those chronic periodontitis patients. After Simple Logistic Regression was conducted, finally, sign value obtained was about 0.258 indicating that IL-1B gene polymorphism did not affect the occurrence of aggressive periodontitis and chronic periodontitis.

PCR-RFLP results of IL-1B (+3954) gene after being cut with TaqI enzyme

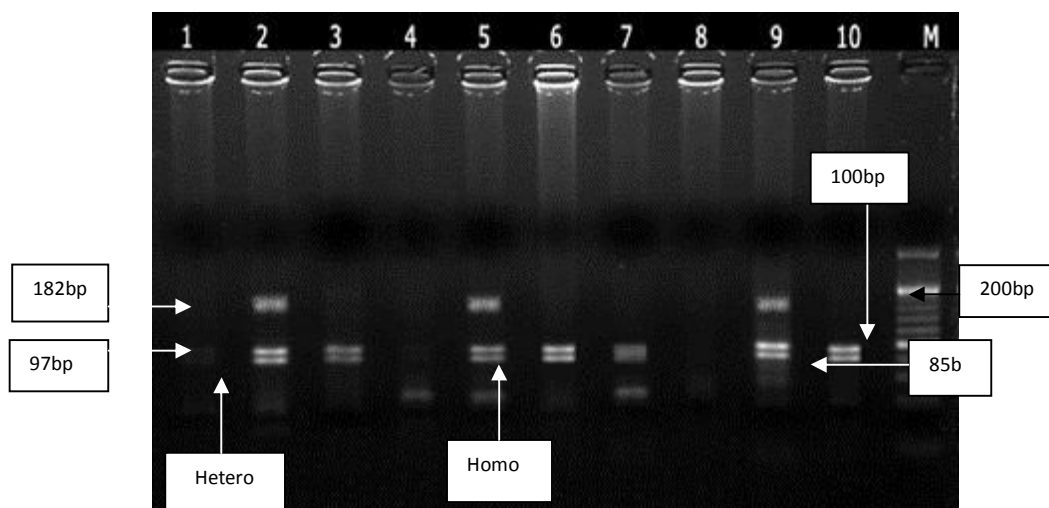


Figure 1: PCR-RFLP Results of IL – 1B (+3954) gene Line 1,4,8 without any gene, Line 2,5,9 with amplicon hydrolyzed at 182bp + 97bp and 85bp (mutant heterozygote), Line 3,6,7,10 with amplicon at 85bp and 97bp (normal homozygote), Line M = DNA marker 20bp.

Discussion

The results suggest that a variety of risk factor may predispose individuals to periodontitis. They also indicate the existence of genetically predetermined high risk groups. Interleukin-1 is one of cytokines that plays a role in inflammation and host defense located on chromosome 2q13-21 with a molecular weight of 415 kb. Interleukin-1 β of cytokine is also involved in the pathogenesis of various diseases, including periodontitis, which does not only have the ability to stimulate fibroblasts and to produce collagenase enzymes, and but also cause periodontal tissue damage¹¹.

IL-1B (3954 +) gene polymorphism in this research, furthermore, had no effect on the occurrence of aggressive periodontitis. Based on the results, it is known that the higher samples suffering from chronic periodontitis was in allele 1 +2 [T] a condition similar to the result of the reserach conducted by Kaarthikeyan *et al*¹², showing that the percentage of T allele in chronic periodontitis group is higher although there was no significant difference. Unlike those results, the result of the research conducted by Parkhill *et al.*¹¹, showed that allele 1 [C] is considered as a variant gene mostly found in population. Meanwhile, allele 2 [T] at (+3954) was indicated as polymorphism associated with severity of periodontitis¹³. It was also known that monocyte derived from patients with mutant homozygote / allele 2 [T] produces more IL-1 β cytokine as many as four times, while heterozygote / allele 1 + 2 produced more IL-1 β cytokine as many as twice than homozygote/ allele 1 [C] where there was a change of C \rightarrow T at +3954 and associated with severity of periodontitis in the Caucasian population¹⁴.

Eskan *et al.*¹⁵ also stated that IL-1 β cytokine is a kind of cytokine potential for inflammation. It is because the variety of cellular functions due to the release processes of IL-1 β cytokine, such as proliferation, activation, and differentiation, also stimulates chemotaxis of leukocyte. The most important thing of the release processes of IL-1 β cytokine, which is the stimulation of various pro-inflammatory cytokines that can exacerbate existing disorders. Meanwhile, Lopez *et al*¹⁶ and Kaarthikeyan, *et al*¹² stated that normal homozygote / allele 1 [C] is considered to be protective from periodontitis, while mutant homozygote / allele 2 [T] is found in small amounts and under the same condition as in this research. Quappe¹⁷ also stated that there was a positive relation of aggressive periodontitis and IL-1B +3954 allele 2 polymorphisms, and normal allele[C] is possibly considered to be protective from periodontitis in the Chile population.

Nevertheless, there was a research of India ethnics conducted by Agarwal *et al.*¹⁸, that supported by showing that IL-1B polymorphism was associated with chronic periodontitis on India ethnics. It was also known that individual vulnerability was likely to included specific genotypes that involves one or more genetic polymorphisms of IL-1B. Thus, it will cause the production of abnormal cytokines, and this increase can cause severe periodontal tissue damage, which in turn can cause a risk of periodontitis.

Arab *et al*¹⁹, and Poulsen *et al.*¹⁰, also had conducted studies of IL-1B polymorphisms suggesting that allele 2 [T] polymorphism was a risk factor for the occurrence of periodontitis, but in this research the variation of allele 2 [T] polymorphism (mutant homozygote) is found only in some samples so that it can be indicated that allele 2 [T] polymorphism (mutant homozygote) is not a risk factor for aggressive periodontitis in Surabaya – Indonesia population.

Therefore it can finally be concluded that the relation of IL-1B (+3954) gene polymorphism in aggressive periodontitis and chronic periodontitis in Indonesian population did not exist. In order to be able to detect polymorphisms in periodontitis more accurately, more research on the relation of the polymorphism and the biological effects with a larger number of samples is needed. Considering that periodontitis is a polygenic disorders, a further and more elaborate research must be conducted to learn about specific polymorphisms in Indonesian population suffering from periodontitis disease.

Conclusion

IL-1B (nt3954) polymorphism did not influence in the occurrence of Aggressive Periodontitis, it was found that patients with IL-1B (nt3954) gene polymorphism had a risk to suffer from Chronic Periodontitis 4.1 times higher

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References

1. Newman MG, Takei N, Klokkevold P, Carranza F. Carranza' Clinical Periodontology 10th ed. WB Saunder 2006; 168- 81,409-414.
2. Hart TC dan Atkinson JC. Mendelian forms of periodontitis. *Periodontol* 2000 2007; 45: 95–112.
3. Levin L, Baev V, Lev R, Stabholz A dan Ashkenazi M. Aggressive Periodontitis Among Young Israeli Army Personnel. *J Periodontol* 2006; 77:1392–1396.
4. Meng H, Xu L, Li Q, Han J dan Zhao Y. Determinants of host susceptibility in Aggressive periodontitis. *Periodontology* 2000 2007; 43:133–159.
5. Loos BG, John RP, and Laine ML. Identification of genetik risk factors for periodontitis and possible mechanisms of action. *J Clin Periodontol* 2005; 32(6):159–179.
6. Passarge E. Color atlas of genetics 3rd Ed. Thieme Stuttgart-New York 2007;170–172.
7. Chen H, Wilkins LM, Aziz N, Cannings C, Wyllie DH, Bingle C, et al. Single nucleotide polymorphisms in the human interleukin-1B gene affect transcription according to haplotype context. *Human Molecular Genetic* 2006;15(4):519-529.
8. Guzeldemir E, Gunhan M, Ozcelik O, Tastan H. Interleukin-1 dan tumor necrosis factor- α gene polymorphisms in Turkish patients with localized aggressive periodontitis. *J of Oral Science* 2008;50(2):151-159.
9. Kowalski J, Gorska R, Dragan M, dan Kozak I. Clinical state of the patients with periodontitis, IL-1 polymorphism dan pathogens in periodontal pocket – is there a link? (An introductory report). *Advances in Medical Sciences* 2006;51:9–12.
10. Poulsen AH, Sorensen LK, Bendtzen K and Holmstrup P. Polymorphisms within the IL-1 gene cluster : effects on cytokine profiles in peripheral blood and whole blood cell cultures of patients with Aggressive Periodontitis, Juvenile Idiopathic Aethritis, and Rheumatoid Arthritis. *J Periodontal* 2007;78: 475-492.
11. Parkhill JM, Hennig BJW, Chapple ILC, Heasman PA, Taylor JJ. Association of interleukin-1 gene polymorphisms with early-onset periodontitis. *J Clin Periodontol* 2000; 27:682–689.
12. Kaarthikeyan G, Jayakumar DN, Padmalatha O, Sheeja V, Sankari M, Ananand B. Analysis of the association between interleukin -1 β (+3954) gene polymorphism and chronic periodontitis in a sample of the south Indian population. *Indian J Dent Res* 2009;20(1):37 - 40.
13. Kratka Z, Bartova J, Otcenaskova M, Janatova J, and Duskova J. Interleukin-1 Gene polymorphisms as Assessed in a 10 year study of Patients with Early onset Periodontitis. *Folia Microbiol* 2007;52(2):183-188.
14. Kornman KS. Intereukin 1 genetics, inflammatory mechanisms, and nutrigenetic opportunities to modulate diseases of aging. *Am J Clin Nutr* 2006; 83: 475–483.
15. Eskin MA, Benakanakere MR, Rose BG, Zang P, Zhao J. Interleukin -1 β modulates Proinflammatory Cytokine Production in Human Epithelial Cells. *Infection and Immunity* 2008;76 (5):2080-2089.
16. Lopez NJ, Jara L, and Valenzuela CY. Association of Interleukin-1 polymorphisms with periodontal disease. *J Periodontol* 2005;76: 234-243.
17. Quappe L, Jara L, Lopez NJ. Association of interleukin- 1 polymorphism with aggressive periodontitis. *J Periodontol* 2004;75:1509–1515.
18. Agrawal AA, Kapley A, Yeltiwar RK, Purohit HJ. Assessmen of single nucleotide polymorphism at IL-1A+4845 and IL-1B+3954 as genetic susceptibility test for Chronic Periodontitis in Maharashtrian ethnicity. *J Periodontol* 2006;77(9):1515 -1521.
19. Arab HR, Afshari JT, Radvar M, Moeintaghavi A, Sargolzaei N, Rigi A. IL-1B +3954 genetik polimorfisme association with generalized aggressive periodontitis in Khorasan- Iran province. *J Med. Sci* 2007; 7(2) 222–227.

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