

Research Report

Molecular detection of interleukin-1A +4845_{G→T} gene in aggressive periodontitis patients

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ABSTRACT

Background: Abundant researches had been conducted based on the clinical and histopathological pathogenesis of aggressive periodontitis. Nevertheless, there were still few researches which based on molecular biology, and especially related to gene polymorphism. This study was done based on IL-1A +4845_{G→T} gene polymorphism in aggressive periodontitis patients. **Purpose:** The purpose of this study was to characterize the generic variation of IL-1A +4845_{G→T} as a risk factor aggressive periodontitis and chronic periodontitis. **Methods:** DNA from patients with aggressive periodontitis and chronic periodontitis was taken determination of IL-1A +4845_{G→T} polymorphism was conducted with PCR-RFLP technique. **Results:** Homozygous allele TT polymorphism was not found in all samples, only allele GG (wild type) and allele GT (heterozygous mutant) were not affect aggressive periodontitis and chronic periodontitis. **Conclusion:** The study showed there was no significant association between IL-1A +4845_{G→T} gene polymorphism and aggressive periodontitis and chronic periodontitis.

Key words: Interleukin-1A +4845_{G→T} gene polymorphisms, aggressive periodontitis, chronic periodontitis, PCR-RFLP

ABSTRAK

Latar belakang: Penelitian tentang patogenesis periodontitis agresif berdasar klinis dan histopatologi telah banyak dilakukan, akan tetapi penelitian berdasar biologimolekuler terutama polimorfisme gen masih sangat jarang dilakukan. Penelitian ini dilakukan berdasarkan pada polimorfisme gen IL-1A +4845_{G→T} pada penderita periodontitis agresif. **Tujuan:** Tujuan dari penelitian ini adalah untuk mengetahui variasi genetik dari IL-1A +4845_{G→T} yang merupakan faktor risiko periodontitis agresif dan periodontitis kronis. **Metode:** DNA dari penderita periodontitis agresif dan periodontitis kronis diisolasi, selanjutnya dilakukan determinasi dari polimorfisme gen IL-1A +4845_{G→T} dengan menggunakan teknik PCR-RFLP. **Hasil:** Pada seluruh sampel penelitian ini tidak dijumpai polimorfisme allele TT (homosigot mutan), yang didapat adalah jenis allele GG (wild type) dan allele GT (heterosigot mutan) yang tidak berpengaruh terhadap periodontitis agresif dan periodontitis kronis. **Kesimpulan:** Polimorfisme gen IL-1A +4845_{G→T} tidak mempunyai hubungan terhadap kejadian periodontitis agresif dan periodontitis kronis.

Kata kunci: Interleukin -1A +4845_{G→T} polimorfisme gen, periodontitis agresif, periodontitis kronis, PCR-RFLP

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INTRODUCTION

Periodontitis is commonly found in society, such as cardiovascular disorders, diabetes, and other complex diseases due to various factors. Periodontitis, however, is an infectious disease commonly found in periodontal tissues in oral cavity caused by various factors and later can cause periodontal tissue damage. It is known that the periodontitis was triggered by bacterial plaque and defense mechanisms of host, so the understanding of the relationship between host and oral bacteria is the basis understanding of the pathogenesis of periodontal disorder.¹ Many studies have reported that smoking, diabetes, and genetic factors can increase the severity risk of periodontitis. Determination of the etiology of severe of periodontal disease continues to be discussed by researchers, who stated that environmental and genetic are factors contributing to the occurrence of periodontitis.²

Many studies showed that genetic factors influence the occurrence of periodontitis. Genetic polymorphism is closely related to the variety of clinical conditions of periodontitis. Based on research conducted, it is known 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.^{1,3} In the United States of America (USA), it is also known that race gives strong influence on the occurrence of periodontitis. Several studies have shown that the occurrence of aggressive periodontitis in African-Americans is higher than in the white race (Caucasoid), and this condition shows that the African-Americans are vulnerable to aggressive periodontitis, about 10%.^{4,5}

Cytokines, Interleukin-1, is a pro-inflammatory protein with cytokine functions, such as as chemotactic factors playing a role in the onset of inflammatory, and as a mediator and regulator of inflammatory responses in the host innate immune system which plays in a number of biological activities, including proliferation, homeostasis, regeneration, reparation and inflammation.^{6,7} Biological effects of IL-1 depend on the number of cytokines that are released at low levels with their main function as local inflammatory mediators. Meanwhile, IL-1 at high levels moves into circulation, and invited endocrine effects. There are three genes that play a role to regulate the production of IL-1: IL-1A, IL-1B, and IL-1 RN. IL-1A gene associated with the production of IL-1 α cytokines is associated with the occurrence of inflammation.⁸ In in vitro study, it is known that there is a direct relationship between IL-1 genotype and the amount of cytokine secreted in the culture macrophages.^{9,10}

IL-1A polymorphism is associated with the severity of periodontitis.¹¹ Based on it, it was necessary to characterize the genetic variation of IL-1A +4845_{G→T} as a risk factor for aggressive periodontitis and chronic periodontitis as a control group, especially in societies in Surabaya because until now there are no data about the characterization of the IL1A gene. This research, therefore, was expected to

reveal the basic pathogenesis of aggressive periodontitis and chronic periodontitis as well as to be able to be used in determining the basic treatments of patients with aggressive periodontitis and chronic periodontitis. Genetic polymorphism of IL-1A +4845_{G→T} was a point mutation at nucleotide number +4845 from guanine becomes thymine.

MATERIALS AND METHODS

This study was considered as an observational analytic study with a case-control study design in patients who suffer from aggressive periodontitis and chronic periodontitis. Further gene variant test was also conducted on IL-1A +4845_{G→T} with PCR-RFLP. The subjects of study were patients with aggressive periodontitis and chronic periodontitis who came to periodontics clinics Dental Hospital Universitas Airlangga have gained Ethical clearance.

The venous blood of all patients with aggressive periodontitis and chronic periodontitis were taken for about 3 ml by an analyst, and then extracted for its DNA. DNA amplification was conducted by using PCR for about 32 cycles. Primer specific sequences of IL-1A +4845_{G→T} used were F: 5'-ATG GTT TTA GAA ATC ATC AAG CCT AGG GCA-3' and R: 5'-AAT GAA AGG AGG GGA GGA TGA CAG AAA TGT-3'. Afterwards, visualization of PCR IL-1A product was conducted by 3% agarose gel and added with 1 μ l ethidium bromide, then it placed in submarine gel agarose electrophoresis apparatus at 100V, 70 MAMP, for 40 minutes, and recorded by using Gel Doc system. Determination of IL-1A +4845_{G→T} polymorphisms was conducted with PCR-RFLP technique by using restriction endonuclease enzymes; Fnu4HI.

Criteria for IL-1A +4845_{G→T} polymorphism were: (a) allele GG: 124 bp + 29 bp (wild type); (b) allele TT: 153 bp (homozygous mutant); (c) allele GT: 153 bp and 124 bp + 29 bp (heterozygous mutant). Restriction endonuclease enzyme incubated at 37°C for overnight in waterbath incubator. Then, digestion fragments of DNA by RFLP were detected by electrophoresis using 3% high resolution of agarose mixed with 1 μ l ethidium bromide and then run on gel electrophoresis at 100 volts for 40 minutes. Finally, the results were read and recorded by using Gel Doc system.

RESULTS

Patients who have abnormalities in this study were majority women with both aggressive periodontitis and chronic periodontitis. Among patients with aggressive periodontitis, 26 subjects were female, and 11 subjects were male. Meanwhile, among patients with chronic periodontitis, 22 subjects were female, and 12 subjects were male (Figure 1), that there was no significant difference

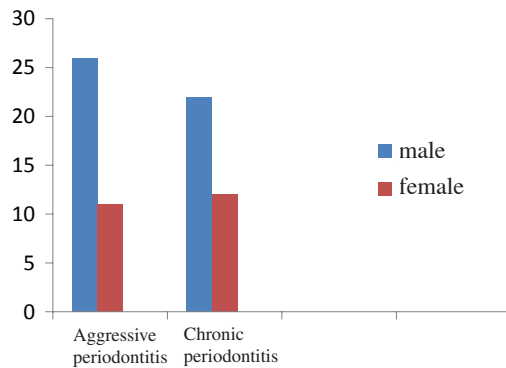


Figure 1. Distribution of patients based on gender.

Table 1. Distribution genotype polymorphism of interleukin-1A +4845_{G→T} gene on aggressive periodontitis

Genotype	n	Frequency %
Allele 1 GG	25	67.5
Allele 1.2 GT	12	32.5
Allele 2 TT	0	0
Total	37	100

Table 2. Distribution alleles of interleukin-1A +4845_{G→T} gene on aggressive periodontitis

Genotype	n	Frequency %
Allele 1 G	62	83.8
Allele 2 T	12	16.2
Total	74	100

Table 3. Distribution genotype polymorphism of interleukin-1A +4845_{G→T} gene on chronic periodontitis

Genotype	n	Frequency %
Allele 1 GG	21	61.7
Allele 1.2 GT	13	38.3
Allele 2 TT	0	0
Total	34	100

Table 4. Distribution alleles of interleukin-1A +4845_{G→T} gene on chronic periodontitis

Genotype	n	Frequency %
Allele 1 G	55	80.9
Allele 2 T	13	19.1
Total	68	100

between male patients and female by statistical test (Chi square $p = 0.821$).

The role of interleukin-1 in periodontal disease, based on in vitro and in vivo studies, can be mentioned as a factor causing degradation of extracellular matrix as well as damage to alveolar bone. It has been mentioned that

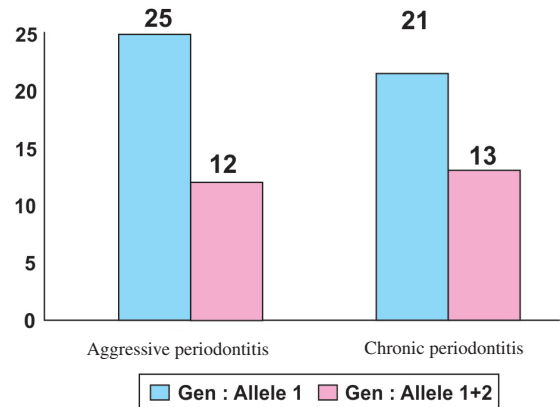


Figure 2. Cross tabulation between the aggressive and chronic periodontitis samples and the polymorphism types.

polymorphism more influenced by racial factors, thus, the results of a study conducted on different races is likely to give different results.

Genotype frequency of IL-1A +4845_{G→T} on aggressive periodontitis was about 67.5% allele GG (wild type), 32.5% allele GT (heterozygote mutant), and no one (0%) allele TT (homozygous mutant). Meanwhile, the frequency of the mutant alleles T in aggressive periodontitis was about 16.2% (Table 1 and 2).

Genotype frequency of IL-1A +4845_{G→T} in chronic periodontitis was 61.7% allele GG (wild type), 38.3% allele GT (heterozygote mutant), and no one (0%) allele TT (homozygous mutant). While the frequency of the mutant allele T in chronic periodontitis was about 19.1% (Table 3 and 4). In this study, we found that allele TT (homozygous mutant) is not found in all samples, but allele GT is the most polymorphism appeared (heterozygous mutant).

Based on Figure 2, it shown that there were difference in the distribution of gene polymorphism in aggressive periodontitis and that in chronic periodontitis. Incidence of alleles GT in patients with aggressive periodontitis was the same as that in patients with chronic periodontitis, nearly about 32.5% and 38.2%. By using Fisher’s Exact test, the significant value obtained was about 0.629. Thus, if α used is about 5%, there will be no different between gene polymorphisms of IL-1A gene +4845_{G→T} in aggressive periodontitis and that in chronic periodontitis.

DISCUSSION

The results of this study showed that based on gender there was no difference between women and men, which means that every individual has the same risk for getting the disease. Based on the number of people, the number of women was higher than that of men probably due to the fact that more women concern with their aesthetic and appearance than men do so that defects in women’s oral cavity will soon be detected earlier. Moreover, periodontal

disorder is known as a disease causing no complaint to the sufferer, and the other possibilities, such as puberty in early age, hormonal changes during menstruation, and pregnancy even can also worsen the clinical condition of periodontitis patients. Another researchers found that the majority of the sample was female, and also found men and women have the same representation in these disorders.^{7,13}

Many studies conducted to examine the role of gene polymorphisms on the host response of patients with periodontitis. Gene polymorphisms that occur in patients with periodontitis are likely to affect protein expression they produce, and will ultimately change the cell morphology and function affecting innate and adaptive immune responses, and possibly manifested in the clinical condition of the patients. Gene polymorphism of IL-1A +4845_{G→T} in this study was type of heterozygous mutant affected on aggressive periodontitis later also affected protein expression. Based on the results of this study, it is also known that gene polymorphisms of IL-1A +4845_{G→T}, allele GT were mostly found in patients with aggressive and chronic periodontitis as disease risk markers. Gonzales *et al.*,¹⁴ did not find gene polymorphisms of IL-1A +4845_{G→T}, but Gulzerdemir *et al.*,⁷ they found few gene polymorphisms of IL-1A +4845_{G→T} allele GT. These results indicate that race factor can influence the characteristics occurrence of gene polymorphisms.

Some studies found that single nucleotide polymorphisms (SNPs) can affect genes encoding pro-inflammatory cytokines, such as IL-1A genes that play a role in chronic and aggressive periodontitis pathogenesis. Number of genotypes and alleles may differ in sick people and healthy ones. Thus, since detected allele can be associated with the disease a research can be conducted to understand role of genes as etiology or a risk factor.¹⁵ Patients with positive mutant genotypes showed statistically lower IgG antibody than those with negative mutant genotype. It indicates that body response to periodontal bacterial pathogens also reduced.¹⁶ It is also known that patients with polymorphisms will have higher bacterial pathogen, and a low antibody titer will against periodontal bacteria.^{17,18}

The response of IL-1A gene polymorphisms with periodontal treatment then reported that patients with an allele TT polymorphism did not show a good response to the treatment using guided tissue regeneration.¹⁹ On the other hand, patients who obtained non-surgical treatment after being evaluated for two years were not associated with genotype polymorphism.²⁰ Therefore, more information and research are needed to understand the biological influences on the clinical effects of IL-1A gene polymorphisms in order to set a more accurate treatment to get better results.

In addition, since periodontitis is considered as multifactorial disease, it requires a lot of data and research to get a clear association between genetic factors, pathogenic process, and clinical condition. However, in this study it can be concluded that there is no difference

found between polymorphism in aggressive periodontitis and that in chronic periodontitis, and also no specific genetic biomarkers for aggressive periodontitis and chronic periodontitis. Further studies with larger samples are needed. Studies about other genes are needed since periodontitis is a polygenic disease that needs control group from a healthy persons (normal group).

ACKNOWLEDGMENTS

This research can be carried out in cooperation between Department of Periodontics, Faculty of Dentistry, Universitas Airlangga and Department of Biochemistry, Faculty of Medicine, Universitas Airlangga, as well as the funding of DP2M of National Higher Education Directorate

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