

Relation of Susceptibility to Periodontitis and Tumor Necrosis Factor Alpha G-308A Polymorphism in Indonesian Males

Windy Najla Rubiati¹, Aisha Zaskia Gani¹, Benso Sulijaya²,
Winoto A Suhartono¹, and Elza Ibrahim Auerkari^{1*}

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

Abstract

Single-nucleotide polymorphism (SNP) in the tumor necrosis factor alpha (TNF- α) gene occurring in the promoter region has been associated with several inflammatory diseases. Periodontitis, an inflammatory disease in tooth-supporting tissue that has a complex multifactorial etiology, affects males more often than females. This study aimed to discover the distribution of TNF- α G-308A polymorphism and its association with periodontitis susceptibility in Indonesian males.

One hundred stored samples of biological DNA extracted from 50 periodontitis patients and 50 healthy controls were analyzed for TNF- α G-308A polymorphism by polymerase chain reaction–restriction fragment length polymorphism. The PCR product was digested with NcoI restriction enzyme.

The AA genotype was absent in both periodontitis patients and healthy controls. GG and GA genotype frequencies were 78% and 22%, respectively, in periodontitis patients, while A and G allele frequencies were 11% and 89%. In healthy people, genotype frequencies of GG and GA were 92% and 8% and the frequencies of alleles A and G were 4% and 96%. No significant differences in genotype or allele distribution were revealed between the groups.

There was no association between TNF- α G-308A SNP and periodontitis susceptibility in Indonesian males.

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Introduction

Periodontitis is the third highest in terms of oral diseases in the world. While this disease affects 53% of the total Southeast Asia population, the same rate is 60% in Indonesia specifically,^{1,2} suggesting that Indonesians have a higher-than-average prevalence rate of periodontitis. Periodontitis is an inflammatory disease found in tooth-supporting tissue with a complex multifactorial etiology.³ The phenotype of this complex disease is determined by both genetic and environmental factors that affect the individual.⁴ Periodontitis is caused by an interaction between anaerobic bacteria in biofilm,

subgingival plaque, and the host's immune response, resulting in the progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both.³ Separately, variations in any number or combination of genes that control the development of the periodontal tissues or the competency of the cellular and humoral immune systems could influence an individual's risk for disease.⁵ Previous research has revealed an association between periodontitis and polymorphisms in certain genes, one of which is tumor necrosis factor alpha (TNF- α), which is related to the host immune response.⁶

TNF- α is located at chromosome 6p21.3 and is one of the most potent proinflammatory cytokines, playing a role in tissue injury and inducing bone resorption in the immune response system.⁷ The cytokine TNF- α has been recorded at high levels in gingival crevicular fluid and gingival tissues from periodontitis lesions.⁸

*Corresponding author:

Elza Ibrahim Auerkari,
Department of Oral Biology,
Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.
E-mail: ei_auerkari@yahoo.com , elza.ibrahim@ui.ac.id

Polymorphisms on the promoter region of the TNF- α gene have a high prevalence in humans. Within the promoter region of TNF- α , a biallelic polymorphic site at position -308 has been reported to influence the production of TNF- α protein.⁹ G-308A (rs1800629) is a polymorphism causing a substitution from guanine (G) to adenine (A) and leads to two- to threefold higher transcriptional activity of TNF- α upon stimulation with bacterial lipopolysaccharide.^{7,10,11}

Carriage of the rare -308 A allele is correlated with significantly greater TNF- α production and transcription and has been associated with an increased risk for the onset of various inflammatory diseases including periodontitis.⁷

A prior meta-analysis by Song et al. suggested that TNF- α G-308A polymorphism confers susceptibility to periodontitis in Brazilian, Asian, and Turkish populations¹²; however, no such results have been collected yet in the Indonesian population. As such, this study aimed to discover the distribution of TNF- α G-308A polymorphism and its association with periodontitis disease susceptibility in Indonesian males.

Materials and methods

Ethical approval

The ethical committee of the Faculty of Dentistry, Universitas Indonesia approved this study. One hundred stored samples of extracted biological DNA from 50 periodontitis patients and 50 healthy control subjects, all male, were used after informed consent forms were signed by each participant.

DNA isolation

A total of 100 DNA samples were compiled, originating from blood serum extracted from patients with periodontitis (50 samples) and healthy controls (50 samples). All samples were stored at -20°C in the laboratory of Oral Biology, Faculty of Dentistry, Universitas Indonesia. The DNA isolation procedure was as reported by Auerkari et al.¹³⁻¹⁶

Polymerase chain reaction (PCR)

An amplification reaction was performed in a 25- μ L reaction mix containing 0.3 μ L of DNA template, 12.5 μ L of KAPA taq ReadyMix PCR with dye, 0.5 μ L of forward primers (5'-AGT CGA CCC AGA TTG TAG GAC AGA GA-3'), 0.5 μ L of

reverse primers (3'-AGG ATC CAG GCA GTT TCC TCT GGA AGG-5'), and 11.2 μ L of ddH₂O. The PCR protocol included a step of 95°C for four minutes, followed by 40 cycles of 95°C for 30 seconds, 56°C for 30 seconds, and 72°C for 15 seconds, with a final extension of 72°C for five minutes. The PCR product (107 bp) was electrophoresed on 1.5% agarose gel at 60 V/400 mA for 30 minutes and visualized using GelDoc.

Restriction fragment - length polymorphism

Next, 10 μ L of the PCR product was digested with 0.25 U of NcoI restriction enzyme (G allele, 87 and 20 bp; A allele, 107 bp), incubated at 37°C for 16 hours, then inactivated at 65°C for 20 minutes using Thermoblock. The generated DNA fragments were analyzed by electrophoresis on a 3% agarose gel at 60 V/400 mA for 30 minutes and visualized using GelDoc.

Statistical analysis

Genotype distributions were compared with those expected for samples from similar populations per Hardy-Weinberg equilibrium. The allele ratio and genotype distribution were analyzed using Fisher's exact test between periodontitis patients and healthy controls with a p-value of less than 0.05 considered as statistically significant.

Results

TNF- α G-308A polymorphism was genotyped in each group of 50 study participants. The genotype frequencies of periodontitis patients and healthy controls were in accordance with Hardy-Weinberg equilibrium.

The distributions of TNF- α G408-A genotype and allele frequencies are presented in Table 1. The AA genotype with one band was absent among both periodontitis patients and healthy controls. Meanwhile, the GG genotype, considered a wild-type homozygote (two bands), and the heterozygote GA genotype (three bands) were observed to some degree in both groups. More specifically, the GG and GA genotype frequencies were 78% and 22%, respectively, in periodontitis patients, while the A and G allele frequencies were 11% and 89%. Among healthy controls, the genotype frequency for GG was 92% and that for GA was 8%, while the frequencies of the A and G alleles were 4% and 96%. The A allele was found more often in the

periodontitis group than in the control group. Using Fisher's exact test, it was determined that no significant differences in either genotype or allele distribution existed between the control and periodontitis groups ($P > .05$).

	Periodontitis patients (n = 50)	Healthy controls (n = 50)	P value
Genotype			
GG	78%	92%	0.0905
GA	22%	8%	
AA	-	-	
Allele			
G	89%	96%	0.0666
A	11%	4%	

Table 1. Genotype and allele frequency distribution of TNF-A G-308A polymorphism.

Discussion

Periodontitis has a complex multifactorial etiology and genetic variations have been reported as contributing factors in many studies. TNF- α is a potent immunologic mediator of acute and chronic inflammatory responses that mediates bone resorption. A variety of single-nucleotide polymorphisms have been identified in the TNF- α promoter and linked with certain chronic inflammatory diseases including cerebral malaria, multiple sclerosis, ulcerative colitis, Alzheimer's disease, chronic bronchitis, and rheumatoid arthritis.⁸ Several case-control studies have been conducted to date to evaluate the role of TNF- α G-308A and periodontitis but the results obtained thus far are largely inconsistent.^{7-9,12}

In this study, we discovered that, although the A allele was more frequently observed than the G allele among periodontitis patients (11%) than healthy controls (4%), no significant difference in either genotype or allele distribution was noted ($P > .05$). These results are in accordance with those obtained by Trevillato et al. (20.7% A allele carriage rate in CP).⁹ However, other research conducted by Dosseva-Panova et al.¹⁷ revealed that the genotype GG of TNF- α G-308A was moderately associated with chronic periodontitis in Bulgarian individuals and a meta-analysis by Song et al. demonstrated that TNF- α G-308A polymorphism confers susceptibility to periodontitis among Brazilian, Asian, and Turkish populations.^{12,18}

The variation among results may be partly explained by the differences in clinical methodological and statistical settings, sample sizes, and TNF genotype distribution profiles within distinct races and/or a single population. The sample size that was used in this study was small, so further research should be conducted with more participants of this population.

Conclusions

Based on our results, the data suggest that TNF-A G-308A polymorphism is not associated with periodontitis susceptibility in Indonesian males.

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Declaration of Interest

The authors report no conflict of interest.

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