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Association of −819 T/C IL-10 gene promoter polymorphisms with susceptibility to leprosy in South Sumatera Indonesia

Desi Oktariana[a], Fifa Argentina[b], Zen Hafy[c], Eddy M. Salim[d], Nova Kurniati[d], Kemas Yakub Rahadiyanto[a] & Evi Lusiana[e]

[a]*Clinical Pathology Department, Medical Faculty, Universitas Sriwijaya, Indonesia*
[b]*Dermatovenereology Department, Medical Faculty, Universitas Sriwijaya, Indonesia*
[c]*Biomedical Department, Medical Faculty, Universitas Sriwijaya, Indonesia*
[d]*Internal Medicine Department, Medical Faculty, Universitas Sriwijaya, Indonesia*
[e]*Pharmacology Department, Medical Faculty, Universitas Sriwijaya, Indonesia*

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Summary
Background: The prevalence of leprosy continues to increase, especially in Indonesia, which is one of the endemic areas of leprosy. One cytokine that plays an important role in the pathogenesis of leprosy is IL-10. Mutations in the gene could cause changes in IL-10 gene expression. One of the most common polymorphisms associated with the IL-10 gene is −819 C/T polymorphism, which is could affect the production of IL-10, and thus influence the process of microbial elimination in the development of leprosy.
Objective: To determine the association of −819 C/T interleukin-10 promoter gene polymorphism in leprosy patients treated at RSUP Dr. Mohammad Hoesin Palembang.
Method: This study was an analytic observational study with a case–control design. Blood from patients who fulfilled the inclusion criteria was taken through venous puncture and stored in EDTA tubes. DNA isolation was carried out with a DNA blood mini kit (QIAamp) and stored at −20 °C. Polymorphism of −819 C/T interleukin-10 promoter gene was measured by PCR-RFLP method, electrophoresis, and visualized under UV light.
Results: In the case group, TT genotype frequency distribution was 21%, CT was 22%, and CC was 7%. In the control group, TT genotype frequency distribution was 28%, CT was 18%, and CC was 4%. In the case group, the allele T frequency distribution was 32% and C allele was 18%. In the control group, the T allele frequency distribution was 37% and C allele was 13%.

Correspondence to: Desi Oktariana, Medical Faculty of Universitas Sriwijaya, Dr. Mohammad Hoesin Hospital, Indonesia, Jln. Dr. Moh. Ali Palembang, Indonesia (e-mail: desioktariana@fk.unsri.ac.id)

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Conclusion: There is no significant association of −819 C/T Interleukin-10 promoter gene polymorphism with susceptibility to leprosy, both in terms of genotypes and alleles of these genes.

Keywords: Leprosy, interleukin-10, polymorphism

Introduction

Leprosy is a disease caused by *Mycobacterium leprae*, an intracytoplasmic parasite of macrophages and Schwann cells, which primarily affects skin and peripheral nerves leading to nerve damage and the development of disabilities.[1] Even though leprosy prevalence has decreased dramatically, the high number of new cases indicates active transmission.[2] The suggestion that disease variation is derived mainly from the host has been raised because of the different clinical manifestations of leprosy, in contrast with the low variability of the bacillus.[3] Previous hypotheses state that the main factor that causes leprosy is the source of transmission, but in some individuals, exposure to *M. leprae* does not cause leprosy.[4] Genetic background can partly explained this fact by the participation of immune response genes, especially cytokine genes.[5]

The unique immune response in each individual is the result of interactions between various cytokine products in the body, which determine the clinical forms of leprosy that develop. The tuberculoid form is the result of high cell-mediated immunity with a largely Th1-type immune response, while the lepromatous form is characterized by low cell-mediated immunity with a humoral Th2 response.[1] Interleukin-10 (IL-10) is one of the cytokines that plays an important role in the pathogenesis of leprosy. It is an important pleiotropic immunoregulatory cytokine mainly secreted by macrophages, but also by T helper lymphocytes, dendritic cells, cytotoxic T cells, B lymphocytes, monocytes and mast cells.[6] Antibody secretion and inflammation can be regulated through exhibition of important immunomodulatory activity by interleukin-10.[7] It not only enhances B-cell survival, proliferation, and antibody production, but also down-regulates the expression of T helper 1 cytokines, major histocompatibility complex class II antigens, and costimulatory molecules on macrophages.[8]

Interleukin-10 gene in human is located within chromosome 1 (1q32) and consists of 5 exons producing a protein of 178 amino acids that functions as a homodimer.[9] Polymorphisms in the promoter regions of IL-10 gene might affect the amount of protein produced.[10] Change in the level of IL-10 production can drive a permissive anti-microbial programming that leads to intracellular *M. leprae* replication. Interleukin (IL)-10 gene polymorphisms at position −819 C/T have been analyzed in several populations demonstrating the association with leprosy, however results of previous works from different populations are variable in Brazilian,[11–16] Malawian,[17] Indian;[18] Mexican;[19] Colombian,[20] and Chinese populations.[21] The aim of this study is to analyze the association of −819 C/T interleukin-10 promoter gene polymorphism with susceptibility of leprosy in Dr. Mohammad Hoesin Hospital, Palembang, Indonesia.

Subject, material, and methods

STUDY POPULATION

A case–control study of outpatients with leprosy from Dr. Mohammad Hoesin Hospital (Palembang, Indonesia) was performed. The case group were diagnosed with leprosy by dermatologists following clinical and histopathological criteria. The classification of leprosy

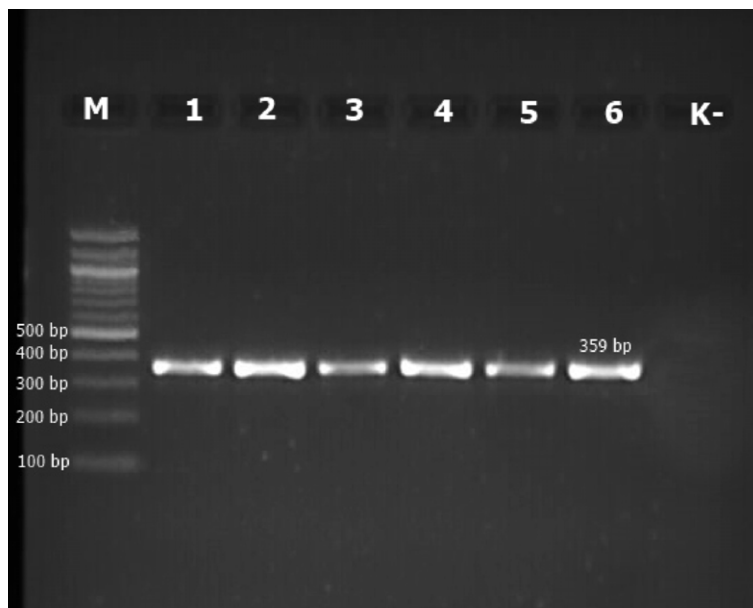

Figure 1. PCR visualization of −819 interleukin-10 promoter gene at 359 bp in 2% agarose gel containing EtBr.

was based on clinical and histological criteria published by WHO. Patients were classified as MB patients (those with a systemic bacterial index > 1) or as PB patients (those with a bacterial index of 0). All patients were treated with multidrug therapy (MDT) specific for MB and PB leprosy, as recommended by the World Health Organization, and MDT was continued throughout the study. The control group consisted of healthy volunteers. General characteristics of subjects are described in Table 1.

This study was approved by the Ethics Committee of Medical Faculty of Sriwijaya University Affiliated with Dr. Mohammad Hoesin Hospital. Healthy volunteers and leprosy patients were recruited and examined at the Dr. Mohammad Hoesin Hospital. Informed consent was obtained from each participant.

BLOOD SAMPLE COLLECTION AND DNA EXTRACTION

Blood samples were withdrawn from a peripheral vein and placed in EDTA-containing tubes (3 mL), then stored at −4 °C for maximum 6 days before cells were separated. The DNA was separated from the patient samples using a QIAamp Blood Kit (Becton Dickinson, Franklin Lakes, NJ, USA) following the manufacturer's instructions. DNA was stored at −20 °C until amplification.

GENOTYPING

For analysis of the polymorphisms present at positions −819 of the IL-10 promoter, PCR amplification of 359-bp DNA fragments was performed using primers 5′-AGACAACACTACTAAGGCTTCTTGAGGA-3′ and 5′-AGGTAGTGCTCACCATGACC-3′, which contained 2 nucleotide mismatch, creating restriction sites for the enzymes *Msl*I (New England Biolabs), respectively. For PCR analysis, 2 µl of genomic DNA was added to a reaction mixture containing 1 µl of specific forward and reverse primers with concentration

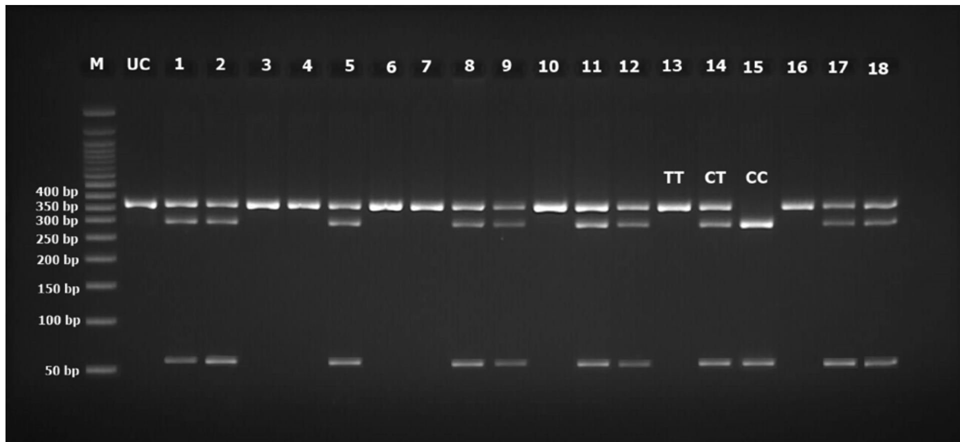

Figure 2. Interleukin-10 gene electrophoresis after being restricted using the *Msl*I enzyme. M = DNA marker, UC = uncut (amplicon which is not cut with *Msl*I enzyme). TT genotype at 359 bp on lines 3, 4, 6, 7, 10, 13 and 16. CT genotype at 65 bp, 294 bp, and 359 bp on lines 1, 2, 5, 8, 9, 11, 12, 14, 17, 18. CC genotype at 65 bp and 294 bp on line 15.

Table 1. General characteristic of subjects

Characteristic	Group		*p*
	Case (50) *n* (%)	Control (50) *n* (%)	
Gender			
Male (*n*, %)	33 (66)	19 (38)	
Female (*n*, %)	17 (34)	31 (62)	0.009*
Age (year)	42.08 ± 14.65	37.78 ± 9.17	0.082**
Ethnic			
Melayu	49 (98)	46 (92)	
Chinese	1 (2)	4 (8)	0.169*
WHO Classification			
Multibacillary (MB)	46 (92)	—	
Paucibacillary (PB)	4 (8)	—	

* Chi-square test, ** independent *t*-test, $p < 0.05$.

of 12.5 pmol, 10 µl of Go Taq PCR mix (Promega, USA), and 12 µl ddH2O. PCR mixtures were incubated initially for 10 min at 95 °C and then submitted to 35 cycles of 94 °C for 30 s, 59 °C for 30 s, and 72 °C for 45 s, followed by an extension cycle of 72 °C for 7 min (Figure 1). Amplified products were digested with *Msl*I, generating fragments of 294 and 65 bp, respectively, and were visualized by electrophoresis in 4% agarose gel and ethidium bromide staining (Figure 2).

STATISTICAL ANALYSIS

For each polymorphism, the percentage of individuals with a particular genotype was calculated separately for cases and controls. The percentage of cases with a particular genotype was also calculated separately for PB and MB cases. Hardy–Weinberg equilibrium was assessed by using the chi-square test for each group. The chi-square test was used to compare

Table 2. Genotype frequency and statistical analyses

Genotype	Case	Control		OR (95% CI)	*p*
TT	21 (42%)	28 (56%)	TT vs		
CT	22 (44%)	18 (36%)	CT + CC	1.758 (0.796–3.880)	0.230
CC	7 (14%)	4 (8%)			

Table 3. Subgroup analysis

	MB vs Control		PB vs Control	
Genotype	OR (95% CI)	*p*	OR (95% CI)	*p*
TT		0.148		0.426
CT + CC	1.980 (0.877–4.468)		0.424 (0.041–4.365)	

Table 4. Allele frequency and statistical analyses

Genotype	Case	Control	OR (95% CI)	*p*
C	35 (38%)	26 (26%)	1.748 (0.946–3.229)	0.102
T	57 (62%)	74 (74%)		

*Chi-Square test with continuity correction, OR = Odds ratio, CI = Confidence interval.

allele and genotype distribution in patients and healthy controls. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. A p value of <0.05 was considered to be statistically significant.

Results

Table 2 shows the genotype frequency and statistical analysis of −819 C/T IL-10 promoter SNPs between leprosy patients and healthy controls. The genotype frequencies for these SNPs were found not to deviate significantly from Hardy–Weinberg equilibrium in patients, nor in controls. Genotype −819 TT is found more commonly in controls (56%) compared to cases (42%). Genotype −819 CT and −819 CC are found more commonly in cases (44% and 14%) compared to controls (36% and 8%). Analysis of −819 C/T SNPs genotypes did not show any significant differences between leprosy patients and controls ($p > 0.05$). In Table 3, the statistical analysis between common and polymorphic genotype in each subgroup of MB and PB did not show any significant difference ($p > 0.05$).

Table 4 shows allelic frequency and statistical analysis of −819 C/T IL-10 promoter SNPs between leprosy patients and healthy controls. Allele T is found more commonly in controls (74%) compared to cases (62%). In the other hand, allele C is found more commonly in cases (38%) compared to controls (26%). Unfortunately, the allelic frequencies of −819 C/T SNPs did not show any significant difference between cases and controls.

Discussion

The association of IL-10 promoter SNPs with susceptibility to leprosy has been demonstrated in Brazilian,[11–16] Malawian,[17] Indian;[18] Mexican;[19] Colombian,[20] and Chinese populations;[21] however, the frequency and distribution of IL-10 SNPs varies by population.[22,23] Because a

similar analysis has not been performed on populations living in South Sumatra, Indonesia, this study sought to compare the distribution of IL-10 promoter polymorphisms, specifically at point −819, in leprosy and healthy patients.

Five studies have shown a relationship between IL-10 −819 C/T polymorphism with susceptibility to leprosy. In a study conducted by Santos in 2002, the frequency of the 819 TT genotype was significantly higher in leprosy patients than controls in the Brazilian population. Interleukin-10 −819 T allele was significantly more common among patients with PB leprosy, in which there was a cell-mediated immune response. In 2004, Moraes found protective haplotypes and haplotypes that were vulnerable to leprosy. The allele −819 C is included in the protective haplotype, while the −819 T allele is one of the haplotypes that is susceptible to leprosy. The research of Pereira[14] and Alvarado-Arnez[11] also stated that the −819 T allele was related to susceptibility to leprosy. The haplotype carrying the −819 T allele also shows susceptibility to leprosy. Carrier −819 T alleles show lower IL-10 production in vitro compared to non-carriers. Malhotra[18] in the Indian population also revealed that the frequency of the 819 T allele and TT genotype increased significantly in leprosy patients.

In contrast to the above statement, several studies conducted in various populations found different results. Research conducted by Franceschi in 2009 found that IL-10 genotype analysis did not show significant differences between leprosy patients and controls. In the Mexican population, Felix's study in 2011 also concluded that there was no association of C819T polymorphisms with lepromatous leprosy in the Mexican population. Furthermore, further research by Garcia in 2013 revealed that the −819 C allele was associated with haplotype protection against leprosy. In Malawi, a study conducted by Fitness in 2004 concluded that there was no relationship between the polymorphism of point −819 and susceptibility to leprosy. In China, research conducted by Chen in 2013 stated that polymorphism of the −819 point promoter IL-10 gene was not associated with susceptibility to leprosy.

This present study did not find an association between −819 C/T polymorphism and susceptibility to leprosy. The cytokine IL-10 has pleiotropic effects in immunoregulation and inflammation, including the inhibition of TH1 cytokine secretion and T cell proliferation.[24,25] Polymorphisms in the promoter affect the amount of IL-10 production. High IL-10 production might increase mycobacterial susceptibility by suppressing inflammation. Interleukin-10 polymorphisms in the distal and proximal regions form haplotypes in gene promoters, and this combination has been associated with cytokine secretion in vitro.[26,27,28] Some polymorphisms in the IL-10 gene promoter area are thought to influence IL-10 production. Several genetic variants of the IL-10 gene promoters, such as A1082G, C819T and C592A, alone or in the haplotype have been widely studied in leprosy patients.[29,30,31]

In contrast with several studies in Brazil, Colombia, and India which state the −819 T allele and −819 TT genotype are alleles that are found in the leprosy population compared with the healthy population, and are associated with susceptibility to leprosy, this study found that the −819 T allele is actually more prevalent in the healthy population compared to the leprosy population, and the −819 C allele is more commonly found in the leprosy population, although statistically the difference is not significant. The results in this study are similar to the results of research in China which found that haplotypes carrying the −819 C allele had a 5.57 times greater risk of being affected by leprosy.[21]

Based on data in the public SNP database (http://www.ncbi.nlm.nih.gov/SNP), the comparison of allele frequencies and genotypes of most of the IL-10 promoter polymorphism in healthy Malay populations differed significantly from Caucasian and African populations. In addition, the frequency of alleles, genotypes, and haplotypes of IL-10 in the case and control

groups differed significantly from other populations, implying genetic heterogeneity between populations. The difference in the frequency of genotypes and haplotypes of IL-10 promoter polymorphisms in various populations might explain the involvement of various genotypes and haplotypes in resistance/susceptibility to leprosy.

In conclusion, this study did not find an association of the polymorphism of the −819 C/T IL-10 promoter gene with leprosy susceptibility in the population of South Sumatra. The heterogeneity between populations observed in leprosy susceptibility may involve other immune regulating genes and environmental factors. The results in this study are consistent with the fact that mycobacterial infection, to have an impact, involves complex interactions between several other host genes and it is also necessary to look at the role of IL-10 in the early and late phases of leprosy infection. The involvement of IL-10 polymorphisms with leprosy in many different ethnic populations suggests that the role of IL-10 needs to be further investigated, both in genetic and functional studies.

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Conflict of interest

No conflict of interest to declare.

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