Identification of IL-10 -819 C / T Gene Polymorphisms in Leprosy

Desi Oktariana1*, Susilawati1, Gita Dwi Prasasty2

1Department of Clinical Pathology, Faculty of Medicine, Universitas Sriwijaya
2 Master’s Program of Biomedical Sciences, Faculty of Medicine, Universitas Sriwijaya

*Correspondence email: deshyfa@gmail.com

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Abstract

Background
Leprosy is a chronic disease caused by an infection of Mycobacterium leprae, which affects skin and nerve lesions, which can result in disability. The prevalence of leprosy continues to increase, especially in Indonesia, which is one of the endemic areas of leprosy. The unique immune response of each individual not only determines the susceptibility of individuals to leprosy, but also determines the type of leprosy that will manifest. One of the immune responses that plays an important role in the pathogenesis of leprosy is interleukin-10 (IL-10). Increased regulation of IL-10 can have an effect on decreasing macrophage activity in killing bacteria. Production of IL-10 is regulated by genes that encode the cytokine. Gen IL-10 is located on chromosome 1q32. Polymorphism in this gene can cause variations in the function of IL-10 protein, which can then influence the process of microbial elimination in the development of leprosy. This study aimed to identify IL-10 -819 C / T gene polymorphisms which were cut with the MslI enzyme in lepers.

Methods
This study is a laboratory study, which is also an observational descriptive study with a cross sectional study approach to 40 subjects with leprosy. Identification of IL-10 -819 C / T gene polymorphism was carried out by PCR amplification and RFLP (restriction fragment length polymorphism) technique using the MslI enzyme.

Results
Distribution of TT, CT, and CC genotypes in the study subjects were 0 (0%), 14 (35%), and 26 (65%). Based on age, the distribution was 0 (0%), 6 (15%), 19 (47.5%) for those under 50 years old, and 0 (0%), 8 (20%), 7 (17.5 %) for those over 50 years old. Based on gender, the distribution is 0 (0%), 5 (12.5%), 16 (40%) in men, and 0 (0%), 9 (22.5%), 10 (25%) in women. Based on the classification of diseases, the distribution was 0 (0%), 3 (7.5%), 7 (17.5%) for papalacillary, and 0 (0%), 11 (27.5%), 19 (47.5 %) for multibasilers.

Conclusion
More wild type genotypes are found in lepers (65%).

Keywords: leprosy, polymorphism, IL-10 gene
Background

Leprosy is a chronic disease caused by an infection of Mycobacterium leprae, which affects skin and nerve lesions, which can result in disability. The prevalence of leprosy continues to increase, especially in Indonesia, which is one of the endemic areas of leprosy. Immune responses that are unique to each individual not only determine the susceptibility of individuals to leprosy, but also determine the type of leprosy that will manifest. One of the immune responses that plays an important role in the pathogenesis of leprosy is interleukin-10 (IL-10). Interleukin-10 has an anti-inflammatory effect, by working on active macrophages to end the response to microbes and restore the system to a state of rest after microbes are destroyed. Increased regulation of IL-10 can affect the decrease in macrophage activity in killing bacteria.

Production of IL-10 is regulated by genes that encode the cytokine. The mutation in the gene is thought to cause changes in the IL-10 gene expression produced. Gen IL-10 is located on chromosome 1q32. Polymorphism in this gene can cause variations in the function of IL-10 protein, which can then influence the process of microbial elimination in the development of leprosy.

Methods

This study was a descriptive observational laboratory study with a cross sectional study approach, to observe and describe the results of observations of the IL-10 gene using PCR-RFLP in lepers. Sampling was carried out in Tanjung Jabung Timur, Jambi. Sample processing includes DNA extraction and PCR-RFLP, conducted at the Biomedical Laboratory of FK Unsri.

Blood samples were taken through 3 ml antecubital venous puncture inserted into a tube containing anticoagulant ethylene diamine tetra acid (EDTA) for DNA extraction and PCR. The PCR process takes place in three repetitive reaction phases of 35 cycles at different temperatures, namely denaturation reaction at 95°C for 10 minutes to separate the double chain into two single chains, annealing reaction which is reuniting the two DNA chains which take place at 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 45 seconds and the extension that
is DNA synthesis through extension of a primer following the paired single chain DNA nucleotide sequence which generally takes place at a temperature of 72°C for 7 minutes.

Detection of IL-10 gene polymorphism was carried out by cutting the DNA of PCR products with MsII restriction enzymes. The existence of a new restriction site can be identified which can be recognized by restriction enzymes. As much as 1 µL of the enzyme was added to the eppendorf tube containing 20 µL of PCR products, then cortexed for a few seconds and incubated in a waterbath at 37°C for 3 hours. After digestion, the PCR product was electrophoresed at 2% agarose gel and was seen by staining ethidium bromide.

**Results**

The age of the research subjects ranged from 20-77 years with the youngest subject being 20 years old and the oldest being 77 years old. The number of subjects aged 50 years and over is 15 people (37.5%), while the number of subjects aged 50 years and under is 25 people (62.5%). Male subjects were more compared to female subjects, namely 21 people (52.50%) men and 19 people (47.50%) women. Persons affected by leprosy with the classification of the paucibacillary number 10 people (25%) and multibacillary 30 people (75%). TT, CT, and CC IL-10 -819 C / T genotypes in the study subjects were 0 (0%), 14 (35%), and 26 (65%) respectively. T and C alleles in the research subjects were 14 (17.5%) and 66 (82.5%), respectively.

Based on age, TT, CT genotypes, CC IL-10 -819 C / T genes in the study subjects were 0 (0%), 6 (15%), 19 (47.5%) for those under 50 years of age, and 0 (0%), 8 (20%), 7 (17.5%) for those over 50 years old. T and C alleles of IL-10 -819 C / T genes in the study subjects were 6 (7.5%) and 44 (55%) for those under 50 years old, and 8 (10%) and 22 (27.5 %) for those over 50 years old. Based on gender, TT, CT genotype, CC IL-10 -819 C / T gene in the study subjects were 0 (0%), 5 (12.5%), 16 (40%) in men, and 0 (0%), 9 (22.5%), 10 (25%) in women. T and C alleles of IL-10 -819 C / T genes are 5 (6.25%) and 37 (46.25%) in men, and 9 (11.25%) and 29 (36.25%) in women. Based on disease classification, TT, CT genotypes, CC IL-10 -819 C / T genes based on the study subjects were 0 (0%), 3 (7.5%), 7 (17.5%) for paucibacillary, and 0 (0%), 11 (27.5%), 19 (47.5%) for multibacillary. These T and C alleles of IL-10 -819 C
/ T genes are 3 (3.75%) and 17 (21.25%) for paucibacillary, and 11 (13.75%) and 49 (61.25%) for multibacillary.

Discussion

The polymorphism that occurs in the IL-10 gene which is cut with the MslI enzyme is in the form of a base C (cytosine) change into a T base (thymine) in the 819th base. The wild type (CC) genotype when visualized will show 1 band, heterozygous (CT) genotypes when visualized will show 3 bands, and mutant homozygous genotypes (TT) when visualized will show 2 bands in the marker area.11,12,13

Changes in C→T in the base of the 819 IL-10 genes can affect the regulation of transcription. Gen IL-10 is located on chromosome 1 in 1q31-32, for about 4.7 kb and containing four introns and five exons. Region 5’ flanking is the DNA region attached to the 5’ end of the gene, which contains promoters and may contain other enhancers or protein binding sites. This region mainly functions in the regulation of gene transcription. Polymorphism in this region can trigger changes in the regulation of transcription. Region 3’ flanking is the DNA region attached to the 3’ end of the gene, which is not copied into mature mRNA. This region contains sequences that affect the 3’ tip formation of DNA, which may also contain other enhancers or protein binding sites.13,14,15

Distribution of TT, CT, and CC IL-10 -819 C / T genotypes in the subjects of this study were 0 (0%), 14 (35%), and 26 (65%), respectively. In this study no mutant homozygous genotype (TT) was found, while the most common genotype was the wild type genotype (CC). Most leprosy patients have a normal IL-10 gene type or wild type (CC). Some researchers report the same thing, as in a study conducted by Chae et al (2008), in a population in Korea, which stated that the TT genotype in IL-10 -819 C/T gene polymorphism was not associated with the risk of leprosy.36 Meanwhile, Yamamori et al. (2004) stated that the TT genotype in the IL-10 -819 C / T gene polymorphism reduced the risk of leprosy.13 On the other hand, research by Bae et al (2008), in the population in Korea, and Hansen et al (2011) stated that TT genotype in IL-10 -819 C / T gene polymorphism increases the risk of leprosy.34,35 Other researchers, namely Lei et al (2011), in populations in China, stated that CT and TT genotypes in IL-10 -819 C gene polymorphism / T increases the risk of leprosy cancer.16,17,18
The allele distribution of IL-10 -819 C / T gene polymorphisms for the C allele was 66 (82.5%) and the T allele was 14 (17.5%). From various studies conducted in several countries both in Europe, America, and several countries in Asia such as Japan and Korea, it turns out that the distribution of C and T alleles from the results of this study is not much different from those studies. Some of these studies include the Hansen et al (2011) study which reported the percentages of C and T alleles 64% and 36%, Lurge et al. (2008), which reported percentage of C and T alleles 66% and 34%, respectively. Yamamori et al. (2004) reported the percentages of C and T alleles of 62% and 38%, and Bae et al. (2008) who reported the percentages of C and T alleles 59% and 41% respectively.\textsuperscript{19,20,21,22}

Patients with TT, CT, and CC genotypes under the age of 50 are 0 (0%), 6 (15%), and 19 (47.5%) respectively, while those aged 50 years and above each are 0 (0%), 8 (20%), and 7 (17.5%). Thus it can be concluded that in this study, the IL-10 gene polymorphism with heterozygous genotypes most often occurs at the age of 50 years and over. As with heterozygous genotypes, wild type genotypes most often occur under the age of 50 years.\textsuperscript{23,24,25}

In this study, C allele was more dominant in the age group below 50 years than in the age group above 50 years, while the T allele was more dominant in the age group above 50 years when compared to the age group under 50 years. However, the difference in the number of T alleles in the two age groups is not significant.

In men, the TT, CT, and TT genotypes found were 0 (0%), 5 (12.5%), and 16 (40%) respectively, while in women each was 0 (0%), 9 (22.5%), and 10 (25%). CT genotypes are more in women, whereas CC genotypes are more in men. Until now, there have been no studies regarding the relationship between genotypes in IL-10 gene and sex polymorphisms.\textsuperscript{26,27}

In this study, C allele was found more in men than in women. As with the T allele which is more commonly found in women. Until now, there have been no studies that state the relationship between alleles in IL-10 gene polymorphisms and sex. The IL-10 gene is a gene that is inherited autosomal not by adherence to genes X or Y.\textsuperscript{13} Thus, it cannot be linked between alleles of this gene with sex.\textsuperscript{28,29}

The highest distribution of CT and CC genotypes is in the multibacillary leprosy group, which is 11 (27.5%), and 19 (47.5%) compared to the pausibacillary which is only 3 (7.5%), and 7 (17.5 %), because the population of the multibacillary classification subjects was greater than the subjects with the pausibacillary classification.\textsuperscript{30}
In this study, the C and T alleles were more dominant in the group of subjects with multibacillary classifications when compared to the passive producers. This is because the number of subjects with multibacillary classifications is far more than the number of subjects with the pausibacillary classification. 28,29,30

Conclusion
Wild type genotypes are more commonly found in lepers (65%).

References


