

DISTRIBUTION OF -819 INTERLEUKIN-10 PROMOTER GENE POLYMORPHISMS AMONG LEPROSY PATIENTS

¹Desi Oktariana, ²Fifa Argentina, ³Zen Hafy, ⁴Eddy M. Salim, ⁴Nova Kurniati, ¹Kms. Ya'kub R

¹Clinical Pathology Department, Medical Faculty, Universitas Sriwijaya, Indonesia.

²Dermatovenereology Department, Medical Faculty, Universitas Sriwijaya, Indonesia.

³Biomedical Department, Medical Faculty, Universitas Sriwijaya, Indonesia.

⁴Internal Medicine Department, Medical Faculty, Universitas Sriwijaya, Indonesia.

Email: desioktariana@fk.unsri.ac.id

ABSTRACT

Leprosy is a chronic infectious disease of *M. leprae* that causes damage to the skin and nerves. Indonesia is one of the countries which is the endemic areas of leprosy, the prevalence of leprosy continues to increase. The immune response not only determines the type of leprosy that will manifest to individual, but also determines an individual's susceptibility to leprosy. One of the cytokines that play an important role in the pathogenesis of leprosy is interleukin-10 (IL-10), which works on active macrophages to end the response to microbes and return the system to rest when the microbes are destroyed. Increased regulation of IL-10 can affect the decrease in macrophage activity in killing bacteria. Polymorphism of -819 is thought to influence the expression of IL-10 genes. This study aims to identify the polymorphism of the -819 of the interleukin-10 promoter gene in leprosy patients treated at RSUP dr. Mohammad Hoesin Palembang. This research is an observational descriptive study with cross sectional design. Polymorphism of the -819 of the interleukin-10 promoter gene was measured by the PCR-RFLP method, electrophoresis, and visualized in UV light. From this study, the frequency distribution of TT genotypes is 42%, CT is 44%, and CC is 14%. The frequency distribution of T allele is 32% and C allele is 18%. Wild type genotype description is more common in leprosy sufferers (80%).

Keywords: leprosy, polymorphism, interleukin-10

1. INTRODUCTION

Leprosy is a chronic infectious disease of *M. leprae* which causes damage to the skin and nerves which can result in disability. Disabilities in leprosy sufferers causes work productivity to decrease so that it greatly affects the quality of life for people with leprosy. Leprosy creates a big stigma in society, so that people with leprosy are often shunned and ostracized by the community which causes psychosocial problems (Direktorat Jendral P2PL Kemenkes RI, 2012).

The prevalence of leprosy continues to increase, especially in Indonesia, which is one of the leprosy endemic areas. The World Health Assembly (WHA) made a resolution on the elimination of leprosy in 2000 by reducing the

prevalence of leprosy to below 1 case per 10,000 population. This resolution in Indonesia is known as Eliminasi Kusta Tahun 2000 (EKT 2000). Although Indonesia has achieved the national elimination target, 14 regions of Indonesia, especially the eastern part, are still areas with a high burden of leprosy with a new case finding rate of ≥ 10 per 100,000 (Depkes RI, 2005).

The previous hypothesis stated that the main factor that causes leprosy is the source of infection (Prawoto, 2008). This opinion is not entirely correct because in some individuals exposure to *M. leprae* does not cause leprosy. This then led some researchers to be interested

in studying the role of the immune response in leprosy (Alter, et al., 2011).

One of the cytokines believed to play an important role in the pathogenesis of leprosy is interleukin-10 (IL-10). Interleukin-10 is an immune system cytokine (immunoregulator) produced by Th2 cells, Th3 cells, monocytes, dendritic cells, eosinophils, mast cells, and keratinocytes. Phagocytosis by macrophages is the main immune response to the elimination of *M. leprae* because the bacteria live intracellularly. Interleukin-10 acts on active macrophages to end the response to microbes and return the system to a resting state after the microbes have been destroyed. Increased regulation of IL-10 can have an effect on decreasing the activity of macrophages in killing bacteria (Sari, et al., 2013).

The IL-10 gene is located on chromosome 1q32. The presence of polymorphisms in these genes is thought to cause changes in the expression of the IL-10 gene produced, which can then affect the process of microbial elimination in the development of leprosy (Cardoso, et al., 2011).

2. METHODS

This research is a descriptive observational laboratory study with a cross-sectional study approach, to observe and present the results of observations on the IL-10 gene using PCR-RFLP in leprosy patients. Sampling was carried out at dr. Mohammad Hoesin Palembang. Sample processing includes DNA extraction and PCR-RFLP, carried out at the Biotechnology Laboratory of Medical Faculty, Universitas Sriwijaya.

Blood samples were taken through 3 ml of antecubital vein punctures and put into a tube containing ethylene diamine tetra acid (EDTA) anticoagulant for DNA extraction and PCR. The sample used in this study were all patients diagnosed with leprosy by doctors and treated at dr. Mohammad Hoesin Palembang for the period January-February 2019.

DNA isolation was carried out using Qiagen DNA extraction kit according to the procedure listed on the kit.

The primary sequences used were 5'-AGACAACACTACTAAGGCTTCTTGAGG A-3 'for IL 10 F (forward) primers and 5'-AGGTAGTGCTCACCATGACC-3' primers for IL 10 R (reverse) primers. The specificity of the two primers was confirmed by 'BLAST' via the official website <http://www.ncbi.nlm.nih.gov>. The confirmation results showed that both primers were specific for IL-10 gene amplification (-819 C/T). The composition of the mixture with a total volume of 25 µl that was used when carrying out PCR consisted of 10 µl of Go Taq PCR mix (Promega, USA), 12 µl ddH₂O, and 2 µl of printed DNA (template), as well as reverse (R) and forward (F) oligonucleotide primers, 0.5 µl each.

The process of DNA synthesis takes place in three repeated reaction stages of 35 cycles at different temperatures, namely the denaturation reaction at 95°C for 10 minutes to separate the double chain into two single chains, the annealing reaction is the reunification of the two DNA chains which takes place at 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 45 seconds and extension, namely DNA synthesis by extension of a primer following its single-chain DNA nucleotide sequence which generally takes place at 72°C for 7 minutes. The quality of DNA amplified by PCR technique was seen using agarose gel electrophoresis technique (2% concentration). The DNA marker 100bp was used as a marker for the size of the DNA bands from electrophoresis. The gel was electrophoresed at 100 volts for 30 minutes.

The IL-10 gene polymorphisms were determined by PCR-RFLP analysis. The IL-10 gene polymorphism detection was carried out by cutting the DNA of PCR products with the restriction enzyme *M*sII. This will cause the formation of new restriction sites that can be recognized by the restriction enzymes. A total of 0.25 µL of enzyme was added to the eppendorf tube containing 15.5 µL of PCR

product, then it was vortexed for a few seconds and incubated in a water bath at 37°C for 1 hour. After digestion, the PCR product was electrophoresed at 3% agarose gel and analyzed by staining with ethidium bromide, using a 50bp DNA marker.

3. RESULT

A total of 50 people with leprosy participated in this study. The frequency of male subjects was 33 people, while women were 17 people. The mean age was 42.08 ± 14.65 years. The frequency of the Malay race is 49 people, while the frequency of the non-Malay race is 1 person. The most frequent type of leprosy (WHO classification) is the multibacillary type (MB), which is 46 people (92%). The frequency of papalibacillary type (PB) was 4 people (8%).

PCR results of the -819 Interleukin-10 promoter gene were seen at the 359 bp position. After the PCR stage is complete, the research is continued with Restriction Fragment Length Polymorphism (RFLP), which is the restriction stage using the MspI enzyme, which is carried out by mixing the PCR product (amplicon) with the MspI enzyme and incubating at 37°C for 1 hour. There are 3 types of genotypes that can be identified from gene polymorphisms, namely wild type genotypes, polymorphic heterozygous genotypes, and polymorphic homozygous genotypes. The wild type genotype is a common genotype that is often found in genes without polymorphisms. This type of genotype is TT. Polymorphic heterozygous genotype is a genotype produced when there is a change from one base T to C so that the resulting genotype is CT. The polymorphic homozygous genotype is the genotype produced when the two bases T to C change so that the resulting genotype is CC.

The polymorphism of the -819 Interleukin-10 promoter of the gene is characterized by a variation in the image of the allele resulting from the digestion of DNA

fragments by the restriction enzyme MspI which looks like this:

- One band appearance at 359 bp shows that there was no cutting of the amplicon DNA fragment by the MspI enzyme in both alleles, which means that the amplicon contains the homozygote wild type TT genotype.
- Two bands appearance at 65 bp and 294 bp show that the amplicon DNA fragment was cut by the MspI enzyme in both alleles, which means that the amplicon contains the polymorphic homozygote genotype type CC.
- Three bands appearance at 65 bp, 294 bp, and 359 bp show that the amplicon DNA fragment was cut by the MspI enzyme in one of the alleles, which means that the amplicon contains a heterozygote polymorphic type CT genotype.

In this study, the frequency distribution of TT, CT, and CC genotypes in the case groups were 21 (42%), 22 (44%), and 7 (14%), respectively. The frequency distribution of the T allele is 64 (64%), the frequency of the C allele is 36 (36%).

Based on age, the genotypes of TT, CT, CC gene IL-10 -819 in the study subjects were 15 (30%), 17 (34%), 6 (12%) for those aged under 50 years, and 6 (12%), 5 (10%), 1 (2%) for those aged over 50 years. The T and C alleles of IL-10 -819 genes in the study subjects were 47 (47%) and 23 (23%) for those aged under 50 years, and 17 (17%) and 6 (6%) for those aged over 50 years.

Based on gender, genotypes of TT, CT, CC gene IL-10 -819 in study subjects were 16 (32%), 12 (24%), 5 (10%) in men, and 5 (10%), 10 (20%), 2 (4%) in women. The T and C alleles of the IL-10 -819 gene were 44 (44%) and 22 (22%) in men, and 20 (20%) and 14 (14%) in women.

Based on disease classification, genotypes of TT, CT, CC gene IL-10 -819 based on study subjects were 18 (36%), 21 (42%), 7 (14%) for multibacillary, and 3 (6%), 1 (2%), 0 (0%) for paucibacillary. The T and C alleles of IL-10 -819 genes were 57 (57%) and

35 (35%) for multibacillers, as well as 7 (7%) and 1 (1%) for paucibacillers.

4. DISCUSSION

In this study, the frequency of men was 33 people (66%) and women were 17 people (34%). Research Felix et al (2011) in Mexico reported the same thing, the frequency of men in the case group was 69% more than 31% of women. Malhotra et al (2005) in India also reported that the frequency of males is 84% more than that of females is 16%. The opposite result was reported by Alvarado-Arnez et al (2015) in Brazil who stated that the frequency of men in the case group was less, 46% compared to 54% women. Cardona-Castro et al in Colombia (2012) also reported that the frequency of males was 28% less than that of women 72%. Research conducted by Bakker in 2006 showed that men have a 22-fold higher risk of developing leprosy than women.

The mean age of the study subjects was 42.08 ± 14.65 years. Research by Garcia, et al. (2015) reported that the mean age was slightly higher in the case group, 43.35 ± 1.83 compared to the control group, which was 38.39 ± 1.64 . The study by Santos, et al (2002) reported that the mean age in the case group was 49.3 ± 7.3 and in the control group it was 47.4 ± 4.1 .

Doull's research in 1945 in the Philippines states that there is a relationship between the risk of getting leprosy clinically with the age of initial exposure, the risk decreases according to the age of exposure, so that age can be a potential risk factor for contact persons to develop leprosy.

In the research subjects, the most frequent type of leprosy (WHO classification) was the multibacillary type (MB), which was 46 people (92%). The frequency of paucibacillary type (PB) was 4 people (8%). Several epidemiological studies state that the incidence of multibacillary type / MB leprosy is higher than that of paucibacillary / PB. In Ethiopia, for example, the incidence of MB leprosy is 90% higher than PB which is only 10% (Ramos, et al, 2012). Other studies have also stated that

there is an increase in new cases of MB leprosy compared to PB type leprosy (Basel, et al, 2014).

The polymorphism that occurs in the Interleukin-10 gene promoter which is cut with the MspI enzyme is in the form of a common allele base change, in this case T (thymine) becomes base C (cytosine) at base 819. Reference to wild type genotypes is taken from a comparison of dominant and codominant models, while references to mutant genotypes are taken from comparisons of recessive models (Madeshiya, et al, 2017). In this study, the most common allele found was the T allele so that the wild type genotype was TT. Wild type (TT) when visualized will show 1 band, heterozygous genotype (CT) when visualized will show 3 bands, and homozygous mutant genotype (CC) when visualized will show 2 bands in the marker area.

Changes in T→C at the 819 base of the IL-10 gene promoter can affect the transcription of mRNA and IL-10 expression. The promoter region is a region related to the 5' flanking region. Region 5' flanking is a region of DNA attached to the 5' end of the gene, which contains promoters and may contain enhancers or other protein binding sites. This region primarily functions in the regulation of gene transcription. Polymorphisms in this region can trigger changes in transcription regulation. Changes in transcription can affect the amount of IL-10 protein produced. (Trifunovic, et al., 2014).

In a study conducted by Santos in 2002, the frequency of the genotype 819TT was significantly higher in leprosy patients than in controls in the Brazilian population. In the study case group, analysis of the frequency distribution of the IL-10 gene promoter allele showed that the -819T allele was significantly more prevalent in PB type leprosy patients, in whom there was a strong cell-mediated immune response. The fact is that several polymorphisms in the proximal region of the IL-10 promoter can be combined to form a haplotype, the most frequently observed being associated with high IL-10 production is the

haplotype -3575T / -2849G / -2763C / -819T / -592A. Therefore, it is possible that this IL-10 haplotype is associated with a patient's susceptibility to developing leprosy. In fact in the Santos study, in the patient cohort, analysis of the frequency distribution of alleles in the IL-10 promoter gene showed that the IL-10 -819T allele was significantly more common among patients with the PB form of leprosy, where there was a cell-mediated immune response.

The same thing was also conveyed in the research of Pereira (2009) and Alvarado-Arnez (2015) which stated that the -819T allele was associated with leprosy susceptibility. The haplotype of the promoter polymorphism carrying the -819T allele also shows susceptibility to leprosy. The -819T allele carriers showed lower in vitro IL-10 production compared with the non-carriers. The study showed that low IL-10 production can lead to the chronic, unprotected response associated with leprosy.

In contrast to the above statements, several studies conducted in various populations found different things. The absence of a relationship between the IL-10 gene promoter -819 point polymorphism and leprosy susceptibility to conclusions in some of these studies. In parts of the African continent, a study conducted by Fitness in 2004 concluded that there was no relationship between the point -819 polymorphism and leprosy susceptibility. In the Mexican population, Felix's research in 2011 also concluded that there was no association of C819T polymorphism with lepromatous leprosy in the Mexican population. In China, research conducted by Chen in 2013 stated that the IL-10 gene promoter -819 point polymorphism was not associated with leprosy susceptibility.

In the Malawian population it was found that the -592 homozygous variant might increase susceptibility to leprosy (Fitness, et al, 2004). Recently, the -819 T variant has been reported to be weakly associated with leprosy susceptibility in Brazil (Franceschi, 2009; Garcia, 2013). These two variants exhibit an associated disequilibrium and usually co-occur

in the haplotypes (-1082A, -819T, -592A) which are associated with increased IL-10 production. In Brazil, homozygosity for the -592A variant was not significantly associated with susceptibility, but there was a trend in this direction. Thus, it is possible that the ATA haplotype could be associated with leprosy susceptibility, but a larger study is needed to assess this.

5. CONCLUSION

Wild type genotypes are more common in people with leprosy.

REFERENCES

- [1]. Alter A, Alcais A, Abel L, Schurr E. 2008. Leprosy as a genetic model for susceptibility to common infectious diseases. *Hum Genet* 123:227-35.
- [2]. Alvarado-Arnez LE, Amaral EP, Sales-Marques C, M. B. Durães S, C. Cardoso C, Nunes Sarno E, et al. 2015. Association of IL10 polymorphisms and leprosy: A Meta-Analysis. *PLoS ONE* 10:9.
- [3]. Bakker MI, Hatta M, Kwenang A, Van Mosseveld P, Faber WR, Klatser PR, Oskam L. 2006. Risk factors for developing leprosy--a population-based cohort study in Indonesia. *Lepr Rev* 77(1):48-61.
- [4]. Basel P, Pahan D, Moet FJ, Oskam L, Richardus JH. Leprosy incidence: six years follow-up of a population cohort in Bangladesh. *Lepr Rev* 85:158 – 169.
- [5]. Cardoso CC, Pereira AC, Marques CS, Moraes MO. 2011. Leprosy susceptibility: genetic variations regulate innate and adaptive immunity, and disease outcome. *Future Microbiol* 6(5): 533–549.
- [6]. Chen XH, Xiong JH, Ning Y, Wen Y, Liu. 2013. IL-10 promoter SNPs and susceptibility to leprosy in ethnic groups from southwest China. *Genetics*

- and Molecular Research 12 (3): 2876-2885.
- [7]. Depkes RI. 2005. Buku Pedoman Nasional Pemberantasan Penyakit Kusta, Cetakan XVII. Jakarta: Direktorat Jendral PPM dan PLP.
- [8]. Eskdale J, Keijsers V, Huizinga T, Gallagher G. 1999. Microsatellite alleles and single nucleotide polymorphisms (SNP) combine to form four major haplotype families at the human interleukin-10 (IL-10) locus. *Genes Immun* 1:151-5.
- [9]. Franceschi DS, Mazini PS, Rudnick CC, Sell AM, Tsuneto LT, Ribas ML, et al. 2009. Influence of TNF and IL10 gene polymorphisms in the immunopathogenesis of leprosy in the south of Brazil. *Int J Infect Dis* 13:493-8.
- [10]. Malhotra D, Darvishi K, Sood S, Sharma S, Grover Ch, Relhan V, et al. 2005. IL-10 promotor single nucleotide polymorphisms are significantly associated with resistance to leprosy. *Hum Genet* 118:295-300.
- [11]. Mastrangelo G, Neto JS, Valentim da Silva G, Scoizzato L, Fadda E, Dallapicola M, et al. 2011. Leprosy reactions: the effect of gender and household contacts. *Mem Inst Oswaldo Cruz, Rio de Janeiro*, Vol. 106(1): 92-96.
- [12]. Mege JL, Meghari S, Honstettre A, Capo C, Raoult D. 2006. The two faces of interleukin-10 in human infectious severity in leprosy. *Genes Immun* 5:592-5.diseases. *Lancet Infect Dis* 6: 557-69.
- [13]. Moet FJ, Meima A, Oskam L, Richardus JH. 2004. Risk factors for the development of clinical leprosy among contacts, and their relevance for targeted interventions. *Lepr Rev* 75:310 – 326.
- [14]. Moraes MO, Pacheco AG, Schonkeren JJ, Vanderborght PR, Nery JA, Santos AR, et al. 2004. Interleukin-10 promotor singlenucleotide polymorphisms as markers for disease susceptibility and disease severity in leprosy. *Genes Immun* 5:592-5.
- [15]. Pereira AC, Brito-de-Souza VN, Cardoso CC, Dias- Baptista IM, Parelli FP, Venturini J, et al. 2009. Genetic, epidemiological and biological analysis of interleukin-10 promotor single-nucleotide polymorphisms suggests a definitive role for -819C/T in leprosy susceptibility. *Genes Immun* 10:174-80.
- [16]. Ramos JM, Martínez-Martín M, Reyes F, Lemma D, Belinchón I, Gutiérrez F. 2012. Gender differential on characteristics and outcome of leprosy patients admitted to a long-term carerural hospital in South-Eastern Ethiopia. *International Journal for Equity in Health* 11:56.
- [17]. Sari N, Amiruddin MD, Amin S, Adam AM, Djamaluddin W, Vitayani S. 2013. Peran Interleukin-2 (IL-2), Interleukin-10 (IL-10) dan Tumor Necrosis Factor- α (TNF- α) Pada Penyakit Kusta. *MDVI* 40(1):35-40.