



The Polymorphism in Interleukin-6-597 G/A Gene and their Levels on Type 2 Diabetic Patients

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Abstract

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BACKGROUND: Interleukin-6 (IL-6) pro-inflammatory cytokines play a role in the pathogenesis of inflammatory reactions in type 2 diabetes mellitus (T2DM). The -597G/A is one of IL-6 gene polymorphisms that are associated with the T2DM risk.

AIM: This study aimed to observe the polymorphism in IL-6-597 G/A gene and their levels on type 2 diabetic patients at Universitas Sumatera Utara Hospital.

MATERIALS AND METHODS: IL-6 -597 G/A gene polymorphisms and levels were done in 80 type 2 diabetic patients. The levels of IL-6 were performed by enzyme-linked immunosorbent assay method. Analysis of IL-6-597 G/A gene polymorphism was done using polymerase chain reaction (PCR) and restriction fragment length polymorphism. The PCR products were cut by FokI restriction enzyme and then visualized with 4% agarose.

RESULTS: This study showed that the genotype frequency of GG and GA was 97.5 % and 2.5 %, respectively; however, no A/A genotype shown in this population. IL-6 levels were higher in GG genotype group compare to GA+AA genotype group, with the association were significant ($p < 0.05$).

CONCLUSION: This study indicated that GG genotype was common genotype in the IL-6-597 G/A gene polymorphism and the polymorphism was significantly with the IL-6 levels.

Introduction

The International Diabetes Federation estimates that the number of people developing diabetes mellitus (DM) in the world will increase from 240 million in 2007 to 380 million in 2025 [1]. In Asia, Indonesia got the third ranks as the highest number of diabetic patients, after India and China [2]. DM is able to kill 3.4 million people in 2004, and 80% of deaths due to DM occurred in developing countries [3], [4].

Nearly 90% of diabetic in the world were type 2 DM (T2DM). T2DM mostly occurs in the adult age, although it has now been found in adolescents [5]. Chronic hyperglycemia in T2DM occurs due to decreased tissue sensitivity to insulin activity or insulin resistance. Insulin resistance is a condition when insulin in blood is unable to phosphorylate the substrate receptors in the target cell so that there is a decrease uptake glucose into the cell [6].

Genetic factors play a role in insulin resistance [7]. It is estimated that 30–70% of the T2DM

risk can be associated to genetics. Recently, the study has identified the candidate cytokine genes involved in the pathogenesis of T2DM, one of it is Interleukin-6 (IL-6). The production of these cytokines occurs through IL-6 gene expression located on chromosome 7p15-p21 [8].

Single nucleotide polymorphisms (SNPs) are changes in one of nucleotide or gene variations that are found by human deoxyribonucleic acid (DNA) sequencing technique [9]. SNPs in IL-6 gene can influence the level of IL-6 protein expression. Several IL-6 gene SNPs in the IL-6 promoter region have been identified such as SNP rs1800797 (-597 G/A or -598 G/A), rs1800796 (-572 G/C), and rs1800795 (-174 G/C) [10]. The -597 G/A is one of IL-6 polymorphisms that are associated with the T2DM risk [11], [12]. Variants of the IL-6-597 G/A gene are GG, GA, AA genotypes, and G and A alleles [8].

Previous studies in several populations showed that the distribution of A allele in IL-6-597 G/A was significantly related to IL-6 activity or IL-6 levels. IL-6 activity in humans is needed in the body's defense

mechanism, immune response, and hematopoiesis. IL-6 is produced by various cells including monocytes, macrophages, fibroblasts, T-helper 2 cells, and endothelial cells [13].

This study aimed to observe the polymorphism in IL-6-597 G/A gene and their levels on type 2 diabetic patients at Universitas Sumatera Utara (USU) Hospital.

Materials and Methods

A total of 80 type 2 diabetic patients were recruited at Endocrinology Polyclinic USU Hospital. All the subjects enrolled must meet eligibility criteria based on the inclusion/exclusion criteria. The inclusion criteria were age 35–79 years, diagnosed with T2DM since 6 months ago, willing to participate as a study subject by signing an informed consent that has been approved by the Faculty of Medicine, USU-Haji Adam Malik General Hospital ethics committee No 447/2019. Subject of the study was excluded if the subject was suffering from malignant diseases, chronic infectious diseases, anemia, pregnancy, and consuming vitamins/supplements.

Fasting glucose examination was done in the morning after the subject underwent fasting 10–12 h using Cobas 6000 analyzer. IL-6 levels were performed by enzyme-linked immunosorbent assay (ELISA) method with the principle of double antibody sandwich with biotin streptavidin, using a commercial ELISA kit (BioLegend). The sensitivity from this kit is 4 pg/ml. The content was read by a microplate reader at a wavelength of 450 nm. Polymorphism analysis was done by the initial stages of isolating the DNA of blood samples from study subjects. Two hundred microliters of blood from 80 patients were isolated using the Promega Extraction Kit. The DNA isolation procedure was carried out according to the protocol contained in the kit.

Isolated DNA was amplified using the polymerase chain reaction (PCR). PCR reaction was done in 25 μ L consist of each 1 μ L forward and reverse primer, 12.5 μ L GoTaq® Green Master Mix (Promega, USA), 2 μ L DNA sample, and 8.5 μ L dH₂O. The primers used refer to previous study [14]. PCR was carried out with an initial denaturation step at 95°C for 4 min, continued with 30 cycles of denaturation at 95°C for 30 s, annealing at 57°C for 30 s, polymerization at 72°C for 30 s, and after final cycle, there is a final polymerization at 72°C for 5 min.

PCR products were analyzed by electrophoresed using agarose 2%. The PCR products were then analyzed through restriction fragment length polymorphism (RFLP) method by cutting DNA gene sequence using FokI restriction enzyme. RFLP products

were analyzed by electrophoresed using agarose 4% at 100 volt for 1 h. Furthermore, electrophoresis results were detected using Gel Doc 1000 (Biorad, USA) to be visualized with ultraviolet light. Homozygote GG genotypes are shown in DNA fragments 525 bp, GA heterozygote is shown 3 bands at 525 bp, 468 bp, and 57 bp, homozygote AA genotype is shown 2 bands at 468 bp and 57 bp.

The results were analyzed using SPSS version 21. Genotype distribution of IL-6 gene polymorphisms was calculated by direct counting and displayed descriptively. The relationship of IL-6-597 G/A gene polymorphisms with IL-6 levels was analyzed using Mann–Whitney U-test.

Results

The study has been conducted on type 2 diabetic outpatient at Endocrinology Clinic USU Hospital. Fasting glucose levels and IL-6 levels of the study sample data are shown in Table 1.

Table 1: Characteristics of studied groups

Characteristics n = 80	Median (Min-Max)
Fasting glucose levels (mg/dl)	196.00(63.00–542.00)
IL-6 (pg/ml)	49.85(8.75–188.00)

IL-6: Interleukin-6.

PCR product of IL-6 gene and RFLP product from FokI enzyme restriction (IL-6-597 G/A) visualization showed in Figures 1 and 2.

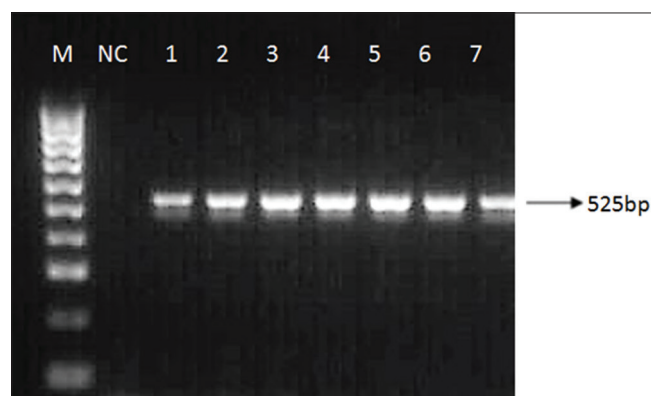


Figure 1: Polymerase chain reaction products analysis of Interleukin (IL)-6 gene visualized in 2% agarose gel electrophoresis. Lane M is a 100 bp DNA ladder, lane 2 is a negative control, and lanes 1–7 represent IL-6 gene (525 bp)

Genotype and allele distribution of IL-6-597 G/A from this population are showed in Table 2.

Table 2: Genotypes and alleles distribution of IL-6-597 G/A

Genotype	N	%
GG	78	97.5
GA	2	2.5
AA	0	0
Allel	N	%
G	158	98.75
A	2	1.25

IL-6: Interleukin-6.

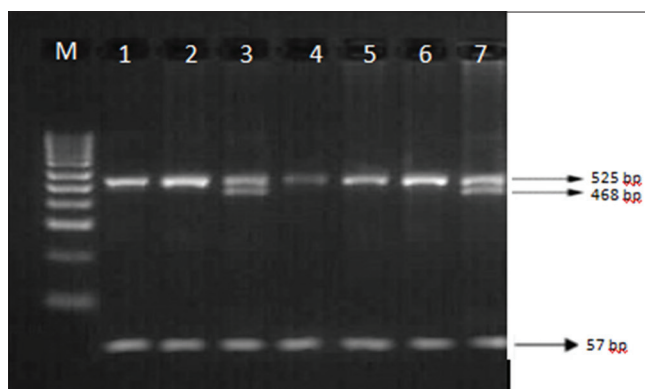


Figure 2: Polymerase chain reaction-restriction fragment length polymorphism of interleukin-6-597 G/A gene polymorphism visualized in 4% agarose gel electrophoresis. Lane M is a 100 bp DNA ladder, lanes 1, 2, 4, 5, and 6 represent homozygote GG (525 bp), lanes 3 and 7 represent heterozygote GA (525 bp, 468 bp, and 57 bp), homozygote AA was not found in this population

Table 3: IL-6 levels found in IL-6-597 G/A genotypes

Genotype	IL-6 (pg/ml)	p-value
GG	41.19 ± 4.4	0.014
GA+AA	13.50 ± 0.1	

IL-6: Interleukin-6.

The result of this study showed that frequency of the GG genotype was higher than GA genotype. However, AA genotype was not found in this study. Table 3 show the IL-6 levels according to distribution of IL-6-597 G/A genotypes. The association between genotypes of IL-6-597 G/A gene polymorphism and IL-6 levels was significant ($p < 0.05$).

Discussion

IL-6 is a pleiotropic cytokine that is involved in the pathophysiology of various human diseases. Human IL-6 protein consists of 212 amino acids with molecular weights ranging from 21 to 28 kDa. The signal peptide structure is composed of 27 amino acids with two potential NH₂-related glycosylation sites. IL-6 is produced by various cells including monocytes, macrophages, fibroblasts, T-helper 2 cells, and endothelial cells [15], [16]. Polymorphism of IL-6-597 G/A is associated with the risk of T2DM [11], [12].

The present study that analyzes IL-6-597 G/A gene polymorphisms has been carried out on 80 patients of type 2 diabetic at USU Hospital, Medan, Indonesia. In this study, GG genotype and G allele showed the highest frequency compared to GA genotype and A allele (97.5% vs. 2.5% and 98.75% vs. 1.25%). However, AA genotype was not found in this population.

The previous study on T2DM patients in Munich, Germany, showed different results, that is, the frequency of GA genotypes was higher than GG and AA genotypes, but the GG genotype was found to be

associated with the incidence of T2DM [11].

Polymorphism IL-6-597 G/A in T2DM populations has not been much studied because the SNPs are rarely found in populations over the world. According to the data of several previous studies, the populations suffering the other diseases (not T2DM) have shown that the distribution of GG genotype of IL-6-597 G/A had the highest frequency in several Asian countries such as China, Korea, and Japan [17], [18]. Same results with the current study, in those Asian countries, AA variants were also not found. In contrary, different results were found that the frequency of GA variants was higher than GG and AA in European countries populations, such as Finlandia, Czech, England, and Germany [19].

The possibility of ethnic differences impacted the occurrence of IL-6-597 G/A gene polymorphisms in populations in Europe and Asia. Genetic polymorphism is the difference in DNA sequence between individuals, groups, or populations. Genetic polymorphisms may be the result of accidental processes, or they may have been induced by external agents influenced by the environment. One type of polymorphism is SNPs, which is the substitution of one nucleotide in a particular sequence in the human genome [9], [19].

Around 3 million common SNPs in the human populations have been identified, of which around 1 million is used to find whether there is an association of SNPs with the susceptibility of diseases such as diabetes and cancer. In some cases, the gene variants can provide evolutionary benefits for species. Variant shape effects can be beneficial and detrimental, depending on the circumstances [20].

The current study only showed the distribution of genotypes and alleles in patients with T2DM, but did not show the role of IL-6-597G/A polymorphism as risk factor for T2DM. Previous studies have shown that polymorphisms IL-6-597 G/A play a role in defense against T2DM. In the previous study, it was found that A allele was associated with a decrease in IL-6 levels [21]. The study of type 2 diabetic at USU Hospital found that IL-6 levels in the GG genotype group were higher than the GA+AA group and a significant association was found between the IL-6-597 G/A polymorphism and IL-6 levels.

Further, the study needs to be conducted to see whether the polymorphism of IL-6-597 G/A and IL-6 levels are associated with the occurrence of T2DM with the study design including a healthy control group.

Conclusion

In this study, it was indicated that the frequency distribution of the IL-6 GG genotype (-597 G/A gene

polymorphism) was higher than the GA variant, and no AA variant was found in this population. These results showed a similarity with several countries populations in Asia that may be due to ethnic similarity. This study also showed that IL-6 levels in the GG genotype group were higher than the GA+AA group and showed a significant association ($p < 0.05$). The IL-6-597 G/A gene polymorphism may be influence the IL-6 levels.

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