The Interleukin-6 (rs1800797) Variant in Healthy Individuals at Medan City, North Sumatera Province

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Abstract

The Interleukin-6/IL6 (rs1800797) variant play a role in several diseases pathogenesis. The frequency genotype of IL6 (rs1800797) varied based on different race, ethnicity, and underlying character in population. This study aimed to assess the frequency of genotype and allele of IL6 (rs1800797) in healthy individuals at Medan city, North Sumatera Province Province and this study can be used as a reference in determining of several diseases for further studies. Genotyping of IL6 (rs1800797) in the promoter region using polymerase chain reaction and restriction fragment length polymorphism method, analysis using FokI restriction enzyme. This study showed that the genotype frequency of GG, GA, and AA were 95.8 %, 4.2 %, and 0 %, respectively. The allele frequency of G and A were 97.9 % and 2.1 %, respectively. The results indicated that the homozygous wild-type (GG) of IL6 (rs1800797) was higher than the heterozygous mutant (GA) and there was absent of homozygous mutant (AA) in this population. The frequency of G allele also higher than A mutant allele.

Keywords: *Interleukin 6 (rs1800797), variant, gene, polymorphism, healthy*

Introduction

The Interleukin-6/IL6(rs1800797) gene variant play a role in several diseases pathogenesis such as febrile seizures, type 2 diabetes mellitus (T2DM), diabetic nephropathy, hyperandrogenism, cancer, and etc.[1-5] IL6 is a pleiotropic cytokine that consists of 212 amino acid with molecular weight ranging from 21 to 28 kDa. The gene of IL6 gene is located on chromosome 7p21, consisting of four introns and five exons, and there are some of very rare polymorphisms in this sequence. The IL6 (rs1800797) gene polymorphism consist of single nucleotide change from G (guanine) to A (adenine) at position -597 in the promoter region. IL6 (rs1800797) gene was found three variant such as homozygous wild-type (GG), heterozygous mutant (GA) and homozygous mutant (AA).[6,7]

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The frequency of the rs1800797 mutant variant varied based on different race and ethnicity in population and character that underlies that population. The previous study of rs1800797 showed that the frequency of homozygous mutant (AA) was not found in the healthy subject of Chinese and Thailand population,[8,9] differ from the genotype frequency of Czech and Swedish population.[10,11] Association of genotype or allele of IL6 (rs1800797) with several diseases also known to vary according to race or ethnicity. In a meta-analysis by Wang et al. (2019) showed IL6 (rs1800797) gene variant may be associated with an increased risk of liver diseases in the non-Asian population.[12] Other meta-analysis showed that cancer diseases such as breast cancer, non-Hodgkin's lymphoma, B-cell lymphoma and diffuse large B-cell lymphoma in Caucasian but not in Asia population were associated with IL6 (rs1800797) gene variant.[5]

Base on genotypes of the IL6 (rs1800797) gene variant may vary according to race or ethnicity on the differences in population and also on the underlying

characters, and the variant genotype may be involved in several diseases, so the present research aimed to find out assess the frequency of genotype and allele of IL6 (rs1800797) in healthy individuals among Medan city, North Sumatera Province. We hope, these results study can be used as a reference in determining of association of IL6 (rs1800797) with several diseases for other next studies by other researchers.

Materials and Methods

Ethics

This study was carried under the ethical provision of the Declaration of Helsinki. The study was approved by the Ethical Committee of Faculty of Medicine, Universitas Sumatera Utara (No. 447/KEPK-FKUSU-RSUPHAM/2019 Informed consent was taken from the participant that agree to participate in this study.

Study Design

This study was an observational with a cross-sectional study design. Healthy subjects in this study were gym participants from several gyms in Medan city, North Sumatera Province, staffs and students in the Faculty of Medicine, USU. Inclusion criteria were male or female, aged 20-65 years, and fasting blood glucose <126 mg/dl. However, subjects who have malignant disease were excluded from this study.

Genotyping of The IL6 (rs1800797)

Analysis of IL6 (rs1800797) variant was carried out in Molecular Biology Integrated Laboratory at Medical Faculty of Universitas Sumatera Utara (USU) from March to December 2019. DNA isolated from leukocyte using isolation kit and DNA purification (Promega, USA).

Polymerase chain reaction (PCR) process in IL6 gene amplification using primer (F) 5'- GGA GTC ACA CAC TCC ACC T -3' (R) 5'-CTGATT GGAAACCTTATTAAG-3.[13] PCR reaction solution consists of primer forward one µL, primer reverse one µL, PCR master mix 12.5 µL, dH2O steril 8.5 µL, DNA template two µL. PCR program carried out with pre-denaturation at 95°C for 4 minutes, then denaturation at 95°C for 30 seconds (30 cycles), annealing at 57°C for 30 seconds, extension at 72°C for 30 seconds, continued by a final extension at 72°C for 5 minutes.[13] PCR product found at 525 bp and continued the digestion by FokI enzyme (ThermoFisher). The RFLP visualization result using UV transilluminator (Gel Doc, BIO-RAD Laboratories USA), were GG genotype (525 bp), GA genotype (525 bp, 468 bp, 57 bp), AA genotype (468 bp, 57 bp).

Statistical Analyses

Frequency of IL6 (rs1800797) variant was displayed descriptively after calculated by direct counting. Distribution of genotypes and alleles were analysis using SPSS program version 22.

Results and Discussion

PCR-RFLP products of IL6 (rs1800797) showed in Figure 1 and 2

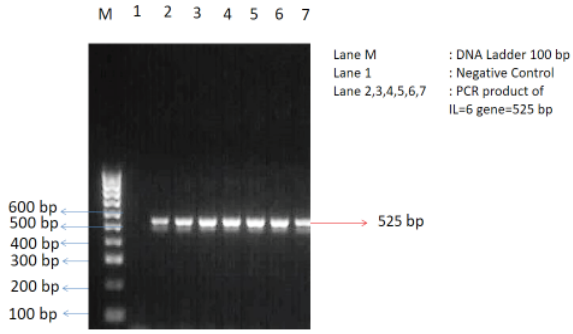

Figure 1
PCR product of IL-6 gene on 2 % agarose gel electrophoresis

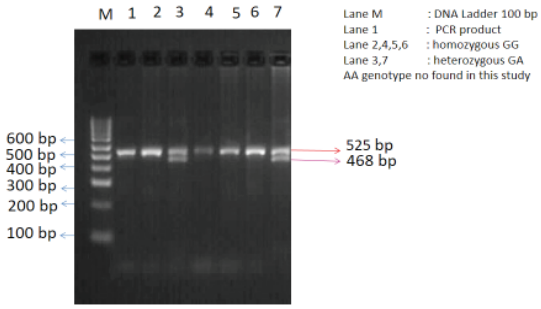

Figure 2. RFLP product of IL-6 (rs1800797) on 4 % agarose gel electrophoresis

IL6 (rs1800797) variant distribution of one hundred and twenty healthy subjects in this study showed in Table 2.

Table 2. Genotypes and alleles distribution of the rs1800797

Genotype	N	%
GG	115	95.8
GA	5	4.2
AA	0	0
Allele	N	%
G	235	97.9
A	5	2.1

The present study showed that the homozygous wild-type (GG) of IL6 (rs1800797) was higher than heterozygous mutant (GA) (95.8% vs 4.2%) and there was absent of homozygous mutant (AA) in this population. The frequency of G allele also higher than A mutant allele (97.9 % vs 2.1 %).

Interleukin-6 (IL-6) is a pro-inflammatory cytokine that has been implicated in various conditions such as cardiovascular disease, diabetes mellitus, etc.[14,15,16] Circulating levels of IL-6 may be influenced by common genetic variants of the IL-6 gene promoter. 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). The IL6 (rs1800797) was found three variants. The genotypes and mutant alleles could be vary based on race and ethnicity of the population of study and the underlying character. Ethnic differences were found in IL-6 (rs1800797) variant.[6,7]

The present study has conducted to analyze the variant distribution of IL6 (rs1800797) in the healthy population at Medan city, North Sumatera Province. Most population in this city is dominated by Batak and Melayu ethnic. This study showed GG genotype of IL6 (rs1800797) was higher than GA genotype (95.8% vs 4.2%). The previous studies in healthy population of Chinese, Tunisia, Brazil, and Egypt showed the same results, i.e. GG genotype higher than GA genotype.[17-21] This present study showed absent of AA genotype. Other previous studies were found the same where the AA homozygote of IL6 (rs1800797) promoter region completely absent in healthy Thailand and Chinese population. [8,9,17]

Our study also showed that G allele was higher than A allele, this was in line with the previous study in healthy population of Thailand, Chinese, Tunisia, Brazil, and Egypt.[9,17-21] But, in India,[22] there was found A allele higher than G allele. Studies in several countries in the world showed the difference in distribution frequency of genotypes (GG, GA, AA) and alleles (G and A) of the IL6 (rs1800797) in the healthy population (Table 3).

Table 3. Genotypes and alleles frequency of IL6 (rs1800797) in healthy control among different populations

Population	Number investigated (N)	Genotypes frequency (%)			Alleles frequency (%)	
		GG	GA	AA	G	A
In this study	120	95.80	4.20	0	97.90	2.10
Chinese [17]	232	100.00	0	0	100.00	0

Cont... **Table 3. Genotypes and alleles frequency of IL6 (rs1800797) in healthy control among different populations**

Tunisia [18]	164	70.75	20.10	9.15	80.80	19.20
Brazil [19]	200	53.50	39.50	7	73.25	26.75
Egypt [20]	102	31.40	67.60	1	65.20	34.80
Egypt [21]	103	31.00	68.00	1	65.00	35.00
India [22]	140	2.86	20.00	77.14	12.86	87.14

To date, experts suggest that several diseases were associated with IL6 gene polymorphism. IL6 (rs1800797) increased the risk of atherogenesis and thrombosis in several diseases, such as coronary artery disease,[23] deep vein thrombosis,[24] acute ischemic stroke.[25] IL6 (rs1800797) also play a role in the pathogenesis of several types of cancer, there were found a significant association between IL 6(rs1800797) variant with breast cancer[26], non-hodgkin's lymphoma[27], but not gastric cancer.[28]

Association of distribution of IL6 (rs1800797) variant with several diseases are known to vary according to race or ethnicity. In the meta-analysis by Wang et al. (2019) showed IL6 polymorphism (rs1800797) may be associated with an increased risk of liver diseases in the non-Asian population.[12] Other meta-analysis showed IL6 (rs1800797) variant associated with several cancer such as breast cancer, non-Hodgkin's lymphoma, B-cell lymphoma and diffuse large B-cell lymphoma in Caucasian but not in Asia.[5] Other study showed that IL6 (rs1800797) associated with T2DM in the German population but did not associated in North India population.[21,22] A study by Phillips et al. (2010) showed that IL6 (rs1800797) associated with metabolic syndrome in French populations, but Boeta-Lopez et al. (2017) showed that there was no association between IL6 (rs1800797) with metabolic syndrome in Mexican-Americans of South Texas population.[29,30]

Further studies are needed in a larger population to analyze IL6 variation based on ethnic and its association with susceptibility of diseases.

Conclusion

The present study concluded that GG genotype of IL6 polymorphism was higher than GA, and there is absent of AA genotype in this population. The frequency of G allele also higher than A mutant allele. This study has differences and similarities with previous studies in other countries, and it was possible because the ethnicity in each country varied. This results study can be used as a reference in determining of association of IL6 (rs1800797) with several diseases for another next study by other researchers.

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Transparency Declaration

Conflicts of Interest: Nothing to declare

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