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The Influence of −174 G/C Interleukin 6 Promoter Gene Polymorphism to Interleukin 6 Concentration in the End Stage Renal Disease Patients with Dialysis: A Single-center Experience in Indonesia

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Abstract

BACKGROUND: Chronic kidney disease (CKD) is classified as a multifactorial disease as a combination of genetic and environmental factors that affect the onset and progression of end-stage renal disease (ESRD). In the last decades are recognized that inflammation, where the critical modulator is cytokines, can occur before the onset of kidney disease and can be a causative factor in the development of CKD. Interleukin (IL)-6 has several polymorphisms in the promoter region, such as 174 G-C, 634 C-G, 572 G-C, and 597 G-A. G/C single nucleotide polymorphism of the IL-6 gene at position 174 in the promoter region is reported to affect the level of IL-6 expression. Unfortunately, there is a lack of data about the genotype frequencies of -174 G/C IL-6 promoter gene polymorphism in Indonesian with ESRD.

AIM: This study aimed to analyze whether −174 G/C IL-6 promoter gene polymorphism influences the concentration of IL-6 in ESRD patients with dialysis in Indonesia.

METHODS: We recruited 95 outpatients who were undergoing regular hemodialysis for ≥3 months at the Rasyida Renal Hospital, Medan, in this cross-sectional study. IL-6 genotype polymorphism was analyzed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The enzyme-linked immunosorbent assay method was performed to assess the quantitative IL-6 serum.

RESULTS: PCR-RFLP examination showed the frequency distribution of the IL-6 genotype -174 G/C gene; 72 (75.8%) respondents had GG genotypes; 22.1% of respondents had the CG genotype, and (2.1%) had the CC genotypes. Patients with the CC genotype were statistically significant to have higher IL-6 concentration compared to other genotypes ($p < 0.001$). Likewise, with diabetic patients, statistics showed higher IL-6 concentration compared to non-diabetics patients ($p < 0.001$).

CONCLUSION: This is the first study showing that -174 G/C IL-6 promoter gene polymorphism influences the IL-6 concentrations in ESRD patients with dialysis in Indonesia. Multicenter studies are needed to validate these findings.

Introduction

The number of patients suffering from chronic kidney disease (CKD) is now increasing in Indonesia. This is a medical, social, and economic problem for patients and their families [1]. CKD is classified as a multifactorial disease, which is a combination of genetic and environmental factors that affect the onset and progression to end-stage renal disease (ESRD). Recent years were recognized that inflammation can occur before the onset of kidney disease and can be a causative factor in the development of CKD [2]. An important modulator in inflammation is cytokines. Increased levels of pro-inflammatory cytokines can be a sign of CKD. Cytokines play an intricate role in the center of coordination in gene activation and suppression. The expression of related factors to inflammatory response is thought to be governed by genetic variations [3].

Variability between individuals in the development of CKD may be due to polymorphisms of various genes that encode cytokines and other inflammatory mediators [4]. These polymorphisms have been found in several studies that are associated with inflammatory and autoimmune disorders [5].

Interleukin 6 (IL-6) is a cytokine with both pros- and anti-inflammatory properties. It is a 21-kDa glycoprotein that is produced by numerous types of cells. The human IL-6 gene is located on chromosome 7p21 and consists of five exons and four introns. IL-6 gene has several polymorphisms in the promoter region; 174 GC, 634 CG, 572 GC, and 597 GA [3]. The most frequently studied polymorphism is single nucleotide polymorphism G/C at position 174 in the promoter region, which has been reported affecting the level of IL-6 expression [3], [5]. This study aims to investigate whether the polymorphism of the IL-6 -174 G/C promoter gene influences the concentration of IL-6 in ESRD patients with dialysis in Indonesia.

Methods

Selection and description of participants

Ninety-five outpatients undergoing regular hemodialysis in the Rasyida Renal Hospital, Medan were included in this cross-sectional study after written informed consent was obtained from each subject. Data were collected from March to September 2019. Patients with an age of younger than 18 years, undergoing hemodialysis for <3 months, had liver disease, active infections, and malignancies were excluded from the study. The study was approved by the Health Research Ethical Committee of Medical School of Universitas Sumatera Utara/H. Adam Malik General Hospital, no. 283/TGL/KEPK FKUSU-RSUP HAM/2019. All procedures in the protocol of this research were performed according to the guidelines of the Declaration of Helsinki.

Technical information

This study uses medical records and interview methods to assess socio-demographic characteristics (e.g., age, sex, diabetes mellitus [DM], hypertension, liver disease, malignancy, and duration of hemodialysis). Height in square meters and weight in kilograms were measured to calculate body mass index (BMI).

Venous blood samples after overnight fasting were collected in plain, ethylenediaminetetraacetic acid and citrate added tubes. The samples were either analyzed immediately or stored at −20 for the −174 G/C test. Routine laboratory methods analyzed calcium and phosphate.

IL-6 in serum was measured with an enzyme-linked immunosorbent assay (ELISA) method using commercial kits (Qayee Bio Vendor IL-6 ELISA, Shanghai). The DNA extraction was examined by polymerase chain reaction (PCR) technique (Esco Swift TM Maxi Thermal Cycler, Singapore). DNA extracted from blood leukocytes from the whole blood taken from the purity and concentration of genomic DNA extract was determined using a spectrophotometer 1000 nanodrop Thermo Scientific (Thermo Fisher Scientific, USA). The PCR- restriction fragment length polymorphism (RFLP) technique was used to detect the polymorphism of the IL-6 -174 G/C promoter gene.

Statistical analysis

Statistical analysis was performed with SPSS software version 22.0 for Windows (SPSS, Chicago, IL, USA). Normal distribution was tested using the Kolmogorov–Smirnov test. Descriptive data for all variables were expressed as mean ± standard deviation (SD), median, and range for continuous data or percent of the group (n) for categorical data. An independent t-test was used to compare serum IL-6 levels among patient characteristics. Genotype frequency and other characteristics of hemodialysis patients between the high and low concentrations of IL-6 were compared using the Chi-square test or Fisher's exact test if required. Prevalence ratios and their 95% confidence intervals were computed to identify the high concentration risk of IL-6 associated with the presence of the C allele. Comparisons were considered significant when ($p < 0.05$).

Results

Characteristics of ESRD patients with dialysis

Most of the patients were male, 53 (55.8%) with the range of age was 22–79 years and have undergone hemodialysis for 43–676 months. The mean ± SD of the BMI was 24.03 ± 4.30 kg/m^2.

On laboratory examination, the mean ± SD of calcium concentration, phosphate concentration and calcium phosphate multiplication were 9.72 ± 0.74 mg/dL, 5.43 ± 0.64 mg/dL, and 52.98 ± 9.08 mg^2/dL^2. Through the ELISA method, the median IL-6 level was 70.1 pg/ml (range 25.4–898.0) with mean ± SD was 97.95 ± 117.93 pg/ml. PCR-RFLP got the frequency distribution of genotypes −174G/C IL-6 gene; 72 patients (75.8%) had the GG genotype; 21 patients (22.1%) had the CG genotype; and two patients (2.1%) had the CC genotype.

Table 1: Hemodialysis patient characteristic based on IL-6 level

Variable	IL-6 concentration		p	PR	95% CI
	High	Low			
Age (years)					
<60	20 (37.0)	34 (63.0)	0.43	1.16	0.81–1.65
≥60	12 (29.3)	29 (70.7)			
Duration of hemodialysis (months)					
<60	13 (34.2)	25 (65.8)	0.93	1.02	0.61–1.72
≥60	19 (33.3)	38 (66.7)			
Diabetes mellitus					
No	18 (24.3)	56 (75.7)	<0.001	3.94	1.77–8.78
Yes	14 (66.7)	7 (33.3)			
BMI (kg/m^2)					
Underweight-normal	26 (38.2)	42 (61.8)	0.14	1.22	0.96–1.55
Overweight-obese	6 (22.2)	21 (77.8)			
Calcium level (mg/dL),					
≤9.5	12 (31.6)	26 (68.4)	0.72	1.06	0.76–1.50
>9.5	20 (35.1)	37 (64.9)			
Phosphate level (mg/dL),					
≤5.5	16 (32.0)	34 (68.0)	0.71	1.09	0.70–1.68
>5.5	16 (35.6)	29 (64.4)			
Polymorphism, n (%)					
Genotype GG	9 (12.5)	63 (87.5)	<0.001	7.10	3.97–12.71
Genotype GC + CC	23 (100.0)	0 (0.00)			

IL: Interleukin, BMI: Body mass index.

The relationship of various factors with IL-6 levels

A receiver operating characteristic curve was used to determine the cutoff value of IL-6 concentration. Based on the area under the curve, the IL-6 cutoff value was 81.1 pg/ml. The characteristics of hemodialysis patients based on the IL-6 concentration category can

Table 2: Comparison of IL-6 level based on hemodialysis patient characteristic

Variable	IL-6 concentration		p
	Mean	Median	
Polymorphism			
CC	656	656.0	<0.001
CG	167.5	118.0	
GG	62.2	62.1	
Age (years)			
<60	95.9	71.3	0.4
≥60	100.6	69.9	
Duration of hemodialysis (months)			
<60	82.3	64.2	0.9
≥60	108.4	71.3	
Diabetes mellitus			
No	94.7	65.4	<0.001
Yes	109.3	92.5	
Classification of BMI			
Underweight-normal	106.1	71.8	0.1
Overweight-obese	77.5	63.1	
Calcium concentration (mg/dL)			
≤9.5	81.6	70.7	0.7
>9.5	108.9	69.9	
Phosphate concentration (mg/dL)			
≤5.5	109.9	70.0	0.7
>5.5	84.7	71.4	

IL: Interleukin, BMI: Body mass index.

be seen in Table 1. Statistical tests significantly showed that the group of patients with high IL-6 concentration (>81.1 pg/ml) was dominated by patients suffering from DM (66.7%) ($p < 0.001$). From this table, we also could conclude that high IL-6 levels are more likely to be experienced 3.94 times (95% CI: 1.77–8.78) by patients suffering from DM. Besides, based on polymorphisms in the IL-6 gene, it can be seen that all patients with C alleles (100%) had a high concentration of IL-6 compared to other groups ($p < 0.001$).

Table 2 showed that patients with the CC genotype were statistically significant to have higher IL-6 concentration compared to other genotypes ($p < 0.001$). Likewise, with diabetic patients, statistics showed higher IL-6 levels compared to non-diabetics patients ($p < 0.001$).

Discussion

Increased levels of IL-6 in patients with ESRD are associated with decreased kidney function, persistent infection, factors associated with dialysis, and genetic factors [6]. Genetic factors have influenced the incidence of ESRD. A research conducted by Aker *et al*. has proved it. His research showed that there is a correlation between IL-6 gene polymorphisms and ESRD. Aker also stated that patients with CC genotype had a higher risk of death than other genotypes [7]. Research conducted by Ng *et al*. regarding the relationship between IL-6 haplotype on human chromosomes and risk factors for kidney dysfunction in patients with type 2 DM found that IL-6 −174 G/C gene polymorphisms are associated with ESRD risk [8]. Ranganath *et al*., in his research, also showed the same thing that the IL-6 gene polymorphism was associated with ESRD risk. His research showed that IL-6 levels increased in ESRD patients, which was a 37.5-fold increase compared to the control group in the CC genotype, 4.4 times in the GC genotype, and 0.18 times in the GG genotype [9].

This study shows different results from studies conducted by Buckham *et al*. [10] and Kandil *et al*. [11] Kandil *et al*. found no difference in IL-6 levels in ESRD patients with patients who were not ESRD ($p = 0.61$) 11. Research conducted by Buchkam *et al*. in Caucasian tribes in various countries showed that GC genotype frequency (48%) higher than the GG genotype (34.8%), then followed by the CC genotype (17.2%). The same study conducted by Kandil *et al*. in Egypt showed that the GG genotype (77.1%) was higher than the GC genotype (20%) and the CC genotype (2.9%) [11]. A study conducted by Ng *et al*. in the United States also found the same results. They showed that the GG genotype (54.6%) was higher than the GC genotype (29.6%) and the CC genotype (15.8%) [8]. Likewise, research conducted by Ranganath *et al*. in India showed that the GG genotype (62.8%) was higher than the GC genotype (31%), followed by the CC genotype (6.2%) [9]. The difference in genotype frequency is possible for various reasons, such as demographic factors of the subject, lifestyle differences, and the number of study samples.

This study shows that patients with the CC genotype ($\bar{x} = 656$) statistically have significantly higher levels of IL-6 than other genotypes such as CG ($\bar{x} = 167.4952$) and GG ($\bar{x} = 62.1653$). The previous studies have suggested that IL-6-174 allele C polymorphism is associated with increased levels of IL-6 in the general population. Patients with IL-6 -174 allele C have higher serum IL-6 levels so that they may have a worse response to treatment [12].

Statistics in this study show that IL-6 levels are higher in diabetic patients than in non-diabetics ($p < 0.001$). Su *et al*. further shows the correlation between IL-6 and autoimmune diabetes. Likewise in type 2 diabetes, IL-6 levels increase and are associated with the development of atherosclerosis [13].

Research conducted by Beberashviilii regarding the relationship between IL-6 and the state of nutritional status proves that there is no relationship between nutritional factors and increased levels of IL-6 [14]. This statement is in line with the results obtained from this study, where the increase in IL-6 levels is not higher in overweight-obese patients (22.2%) compared to underweight-normal patients (38.2%).

Conclusion

This study shows that there is an influence of the 174 G/C IL-6 gene polymorphism on IL-6 concentrations in ESRD patients with dialysis in Indonesia.

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