Genetic Variation of a −176g>c Interleukin-6 Correlated with White Blood Cells Count in Obesity of Indonesia

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Abstract

BACKGROUND: Obesity is a risk factor for cardiovascular disease. Obesity can trigger inflammation by increasing the synthesis of interleukin-6 (IL-6) which leads to leukocyte recruitment, differentiation of B lymphocytes, activating T lymphocyte cells, and triggering hematopoiesis. Variations in the IL-6 promoter gene are known to affect transcription rates. The previous studies have still found different results regarding the relationship between these gene variations and blood cell numbers.

AIM: This study aimed to determine the influence of IL-6 gene variation with white blood cells counts in obese people of Indonesia.

METHODS: A total of 148 people participated in this cross-sectional study. The number of obese people was 80 subjects and there were 68 non-obese people as controls. Obesity was determined based on the criteria of Asians. Results: Genotype CC was not found in this study, while the GC genotype was found only in the obese group. The GG genotype in obese group had waist circumference, diastolic blood pressure, and higher lymphocyte rates significantly higher than the non-obese group (p < 0.05).

CONCLUSIONS: Variation of −174G>C IL-6 gene is associated with high white blood cell counts, especially lymphocytes number in obese people in Indonesia.

Introduction

The prevalence of overweight and obesity continues to increase in the world, including in Indonesia. Overweight and obesity have been associated with an increase of disability and death from cardiovascular diseases [1], [2]. Weight gain plays a role in the pathophysiology of diabetes mellitus, insulin resistance, dyslipidemia, hypertension, and atherosclerosis, due to excessive secretion of adipokines. The rise of white adipose cells can trigger the release of pro-inflammatory cytokines such as interleukin-6 (IL-6) which causes local inflammation and contributes to inflammation in all parts of the body. This inflammation ultimately causes dysfunction in some organs such as the pancreas and liver [3].

IL-6 has an important role in inflammatory, hematopoiesis, immune response, and defense mechanisms [4]. IL-6 in the bone marrow increases the maturation of megakaryocytes so that many blood cells are released into circulation [5]. In addition, IL-6 also increases hematopoiesis, including the synthesis of neutrophils, and causes the mobilization of neutrophils to the site of infection to parasitize pathogens. This cytokine induces differentiation of the T-helper 17 which serves to improve neutrophilic response [6]. Inflammation in the blood vessels and lipoproteins held in the endothelial wall is risk factors for atherosclerosis due to the formation of thrombus [7].

Variations of the −174 G>C IL-6 gene (rs1800795) are widely studied because it affects the transcription of IL-6 genes [5] so it is related to serum IL-6 levels, although there are still differences in results in some countries [8]. Research on the relationship of variations in this gene with blood cell numbers is still contradictory [9], [10]. This study aimed to determine the relationship between the −174G>C IL-6 gene variation with the number of blood cells.

Samples and Methods

This cross-sectional study involved 148 subjects of Javanese ethnicity in Indonesia [11]. The study subjects were grouped into obesity and the non-obese groups. Obesity was determined from the body mass index (BMI) value calculated from the weight (kg) divided by the squaring of the height.
Measurement of anthropometry, blood pressure, and hematological parameters

The study subjects were measured for their height and weight to determine BMI, waist circumference, and blood pressure with calibrated tools. Blood samples were taken for hematological examination (leukocytes, neutrophils, lymphocytes, and platelets) and for DNA isolation. Hematological measurement used an automated hematology meter (Sysmex KX-21NR).

Gene determination by polymerase chain reaction (PCR)-restriction fragment length polymorphism

DNA samples obtained were amplified using primer (forward 5'- TGACTTCAGCTTTACTCTGT-3' and reverse 5'-CTGATTGGAAACCTTAAG-3') with denaturation temperature of 95°C for 1 min, annealing at 55°C for 1 min, and primary extension at 72°C for 1 min in 30 cycles [13]. PCR products were then separated on 2% agarose gel and read with ultraviolet (UV) light (Figure 1).

PCR products were digested with NlaIII enzyme at a temperature of 37°C for 3 h. CC genotype was shown in fragments 122, 45, and 31 base pairs. Genotype GG is shown in fragments 167 and 31 base pairs, while GC genotype was shown by fragments 167, 122, 45, and 31 [14]. The digestion products were separated in 3% agarose gel and read under UV light (Figure 1b).

Statistical analysis

The normality of the data was tested with Kolmogorov–Smirnov. When the data were normally distributed, the independent sample t-test was used. When the data were not normally distributed, the data were analyzed with Mann–Whitney U-test.

Table 1: Characteristics of subjects

| Characteristic                | Obese (n = 80)       | Non-obese (n = 68) | p-value |
|------------------------------|----------------------|--------------------|---------|
| Age (year)                   | 43.07 ± 10.68        | 49.63 ± 12.66      | 0.001*  |
| Female (%)                   | 48 (60)              | 31 (45.6)          | 0.079   |
| Waist circumference (cm)     | 94.64 ± 8.82         | 80.22 ± 6.81       | 0.000*  |
| Systolic blood pressure (mmHg)| 122.63 ± 19.57       | 118.09 ± 15.57     | 0.125   |
| Diastolic blood pressure (mmHg)| 81.50 ± 12.48       | 76.98 ± 9.47       | 0.014*  |
| Leukocyte (×10^3/mm³)        | 7.42 ± 1.7           | 7.03 ± 1.49        | 0.154   |
| Neutrophil (×10^3/mm³)       | 5.21 ± 0.08          | 4.40 ± 1.24        | 0.202   |
| Lymphocyte (×10^3/mm³)       | 2.40 ± 0.66          | 2.17 ± 0.65        | 0.033*  |
| Thrombocyte (×10^3/mm³)      | 302.56 ± 83.75       | 294.72 ± 69.46     | 0.541   |

*Significant with Student's t-test and **significant with Chi-squared test with p < 0.05.
The distribution of −174G>C IL-6 genotype and allele in the obese and non-obese groups found only two people with GC genotypes in the obese group and no GC genotypes were found in the non-obese group (Table 2).

Table 2: Distribution of −174G>C IL-6 genotype and allele in obese and non-obese group

| IL-6 gene variations | Obese (n=80) (%) | Non-obese (n=68) (%) | p-value |
|----------------------|------------------|----------------------|---------|
| Genotypes           |                  |                      |         |
| GG                   | 78 (97.5)        | 68 (100)             | 0.500*  |
| GC                   | 2 (2.5)          | 0                    | 0.179   |
| CC                   | 0                | 0                    | 0.001*  |
| Alleles              |                  |                      |         |
| G                    | 158 (53.38)      | 136 (45.95)          | 0.002*  |
| C                    | 2 (0.67)         | 0                    | 0.85**  |

*Fisher’s exact test. IL-6: Interleukin-6.

The average number of platelets, leukocytes, lymphocytes, neutrophils, age, and waist circumference in the GG and GC genotypes in the obese group did not differ significantly (p > 0.05) (Table 3).

Table 3: Relationship of −174G>C IL-6 gene variation with platelet, leukocyte, and lymphocyte numbers in obese patients

| Variable                        | Obesity (GG, n=78) | Obesity (GC, n=2) | p-value |
|---------------------------------|--------------------|-------------------|---------|
| Age (year)                      | 43.93 ± 10.6       | 44.47 ± 18.64     | 0.85*   |
| Waist circumference (cm)        | 94.57 ± 8.99       | 98.5 ± 3.53       | 0.53*   |
| Systolic blood pressure (mmHg)  | 122.88 ± 19.74     | 112.0 ± 3.35      | 0.45**  |
| Diastolic blood pressure (mmHg) | 81.54 ± 12.54      | 80 ± 14.14        | 0.89*   |
| Leukocyte (x10³/mm³)            | 7.39 ± 1.74        | 3.35 ± 3.18       | 0.45    |
| Neutrophil (x10³/mm³)           | 5.19 ± 5.14        | 5.8 ± 2.5         | 0.39**  |
| Lymphocyte (x10³/mm³)           | 4.0 ± 0.67         | 2.35 ± 0.49       | 0.89**  |
| Thrombocyte (x10³/mm³)          | 300 ± 82.49        | 400 ± 102.53      | 0.94    |

*Significant with Student’s t-test. **significant with Mann–Whitney U-test.

The averages of waist circumference, diastolic blood pressure, and lymphocyte count were significantly higher in the GG genotype in the obese group than in the non-obese and statistically different (p < 0.05) (Table 4).

Table 4: Comparison of platelet, leukocyte, and lymphocyte count between GG genotype of −174 G>C IL-6 gene in obese and non-obese groups

| Variable                        | Obesity (GG) | Non-obesity | p-value |
|---------------------------------|--------------|-------------|---------|
| Age (year)                      | 43.93 ± 10.6 | 49.68 ± 12.68 | 0.005* |
| Waist circumference (cm)        | 94.57 ± 8.99 | 80.22 ± 6.8  | 0.000* |
| Systolic blood pressure (mmHg)  | 122.88 ± 19.74| 118.09 ± 15.57 | 0.176  |
| Diastolic blood pressure (mmHg) | 81.54 ± 12.54 | 76.98 ± 9.46 | 0.012** |
| Leukocyte (x10³/mm³)            | 7.39 ± 1.74   | 7.03 ± 1.49   | 0.179  |
| Neutrophil (x10³/mm³)           | 5.15 ± 5.14   | 4.4 ± 1.24    | 0.228  |
| Lymphocyte (x10³/mm³)           | 2.40 ± 0.67   | 2.17 ± 0.65   | 0.019**|
| Thrombocyte (x10³/mm³)          | 300 ± 82.49   | 294.72 ± 69.46| 0.87   |

*Significant with Student’s t-test. **significant with Mann–Whitney U-test.

**Discussion**

Research about polymorphism of −174G>C IL-6 gene in this study found GG and GC genotypes in the obesity group but only found GG genotype in the non-obese group. The frequency of −174 G>C IL-6 genotype among Asian countries also gave significant results. The mutant CC genotype was not found in population studies in Malaysia but was found in Indian populations, whereas in the Chinese population, no allele C was found [16]. In line with this study, no CC genotypes were found in the ethnic Javanese population of Indonesia. GC heterozygous genotypes were found only in the obese group but only 2.5%, smaller than those reported in the Malaysian (7%) and Indian (30%) populations. Obesity plays a role in inflammation, through the release of pro-inflammatory cytokines such as IL-6 due to increase white adipose tissue [17]. This variation of the IL-6 gene may affect the transcription of gene that affects the number of circulating IL-6 [18].

In the obese group, blood pressure and lymphocyte count in GG genotypes were higher than those of the GC genotype. For the average waist circumference, leukocytes, neutrophil, and platelet count were higher in the GC group. However, the results found no significant difference in the statistical analysis. The results of this study correspond to the study of Ma et al. [19] that found the −174 G>C IL-6 gene variation is not associated with hypertension. The IL-6 gene variation was found to be associated with hypertension in SNP −572 C>G in the Asian population. Byrne et al. [9] obtained similar results to this study which is that there is no link between −174G>C IL-6 gene variation and white blood cell count. However, Fernandez-Real et al. [20] showed conflicting results. The platelet count in C allele −174 G>C IL-6 gene was lower than that of allele G.

Genotype of GG in the obesity group was 97.5% while in the non-obese group, it was 100%. Comparing the GG genotype in the obese and non-obese groups, there were significant differences (p < 0.05) in age, waist circumference, diastolic blood pressure, and lymphocyte numbers. Fat distribution in the abdomen is measured using waist circumference [21]. Central obesity is a risk factor for atherosclerosis by various mechanisms, one of which is through inflammation [3]. Obesity is the result of an imbalance between energy consumption and the amount of energy intake. This IL-6 cytokine increases energy use through increased heart rate, norepinephrine levels, and sympathetic nervous system stimulation [22].

Diastolic blood pressure and lymphocyte count in this study were higher in the genotype group with obesity than non-obese. IL-6 secreted adipose tissue in obese individuals has many roles that increase the level of acute-phase protein in the liver, induce the growth and differentiation of B lymphocyte cells, activate T-system lymphocyte cells, trigger hematopoiesis, differentiate macrophages and dendritic cells, recruit leukocytes, improve neutrophil response, increase megakaryocyte maturation, and trigger platelet release [4], [5], [6]. Continuous chronic inflammation in obesity increases the concentration of IL-6 cytokines, triggering increased production of C-reactive protein (CRP) by the liver. Increased CRP triggers decreased vasodilation and increases vascular damage. In addition, IL-6 activates STAT signaling pathways that can increase NADPH oxidase and eNOS with nitric oxide decrease and vascular superoxide enhancement. This leads to increased vascular permeability, immune cell recruitment, and endothelial dysfunction [23].

Serum
IL-6 is known to be higher in GG genotypes than CC genotypes. GG genotype is associated with increased transcription of IL-6 genes and the mRNA IL-6 level in vitro [17]. Obesity and IL-6 play a role in inflammation so that the presence of both variables is related to waist circumference, blood pressure, and lymphocyte numbers. Platelet numbers in the GG genotype with obesity are higher than non-obese, but statistically were not different significantly. One limitation of this study is the small number of samples prevents concluding the direct or indirect relationship of obesity with the number of cells in the blood count as a marker of inflammation.

Conclusions

This research concluded that variations in the −174 G>C IL-6 gene in the Javanese population found in the GG genotype in obesity are associated with higher diastolic blood pressure and lymphocyte count. This research needs to be continued by examining other genotypes that may affect inflammation due to obesity with a larger number of samples.

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