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Full Length Research Paper

Susceptibility of Lys656Asn Polymorphism of Leptin Receptor Gene to Hypertension in Obese Javanese subjects of Indonesia

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Hypertension is a multifactorial disease influenced by environmental and genetic factors. Most obese subjects exhibit leptin resistance and increase in adiposity. Leptin has the capacity to increase sympathetic nerve activity (SNA) and further increase blood pressure by binding to leptin receptors. There are polymorphisms of the leptin receptor gene. Lys656Asn polymorphism of leptin receptor (*LEPR)* gene results from basic amino acid (Lys) to amide amino acid (Asn) substitution. It influences the occurrence of obesity and hypertension. One hundred and six (106) healthy subjects were recruited for this study and were divided into two groups: obese and control groups comprising of 53 subjects each. Non-fasting blood samples were taken to determine the leptin level by the Elisa method and Lys565Asn polymorphism of LEPR gene was determined by PCR-RFLP method. The t-test was then performed to determine the differences in the phenotypic and leptin levels in all groups. This study showed an increase of leptin level significantly in obese group than control. The obese subjects with high blood pressure have shown higher leptin level than control. Lys656Asn genotype subjects showed more elevated blood pressure than Lys656Lys genotype in the obese group. Lys656Asn genotype subjects in obese group were greater in waist circumference, % body fat and leptin level than in control subjects. In conclusion, Lys656Asn polymorphism of leptin receptor gene is a risk factor for the increase of leptin level and hypertension. This study can be continued by determining some genetic factors related to hypertension and obesity in some ethnic of Indonesia.

Keywords: Susceptibility; Hypertension; *LEPR gene*; obesity; polymorphism

INTRODUCTION

Hypertension is one of the world's major health problems. It is an established risk factor for cardiovascular disease along with the other metabolic syndromes, such as obesity, dyslipidemia, and diabetes (Masuo, 2010). WHO statistics in 2013 showed that the prevalence of raised blood pressure among adults aged ≥ 25 years in percentage (%) in male and female of the year 2008 data were 32,5 and 29,3, respectively (World Health Organization (2013).

Leptin is a hormone derived from adipocyte tissue, which has a function in food intake and energy expenditure. Leptin acts via binding with leptin receptor (LEPR) genes mainly in the hypothalamus and also in the peripheral tissues. Hyperleptinemia and LEPR genes status in obese population is indicative of the leptin-resistant state. It is believed to be the reason for increased risk of obesity-related cardiovascular disease

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by producing disruption in metabolic and inflammatory injury in liver, pancreas and the heart (Komsu-Ornek, 2012; De Oliveira *et* al., 2013). Sympathetic nerve system activity, especially renal sympathetic nerve system increases in obese subjects (LI *et al*., 2015) and this condition is hypothesized to increase of blood pressure in obese subjects (Aizawa-Abe *et al*., 2000).

The genomic sequences of genes encoding leptin and LEPR are polymorphic, and many polymorphisms in it have been reported. The genetic association between polymorphisms and hypertension has been investigated multiple times, but the results are still inconsistent (Liu *et* al., 2000; Suriyaprom *et* al., 2014; Pena *et* al., 2014). In this study, we investigated the polymorphisms of Lys656Asn (G>C) *LEPR* gene (rs8179183) to find the association of this *LEPR* gene with hypertension and obesity in Javanese population of Indonesia.

SUBJECTS AND METHODS

Study Population

A total of 106 participants were screened in this case-control study with the age range of 17-35 years old. Among them, there were 53 healthy controls (40 male, 13 female) and 53 obese subjects (38 male, 15 female). Most of the participants were college students who were screened at the Medical Faculty of the Gadjah Mada University, Yogyakarta. We obtained anamnesis (medical history) and performed physical examination on all subjects. Subjects were excluded from the study if they had history of cardiovascular, metabolic, lung, kidney, and liver diseases. Subjects who were included in the study, were non-smokers, those with no history of corticosteroid drug consumption, had not taken drugs for gaining or losing weight and had a normal three times meal a day with no routine exercise (less than 2-3 times weekly). This study was approved by Ethical Committee Faculty of Medicine, the Gadjah Mada University.The work has been carried out under The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans.

Hypertension was defined using the blood pressure (BP) classification of the eighth report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 8). Normotension is defined as systolic blood pressure (SBP) < 120 mmHg and diastolic blood pressure (DBP) < 80 mmHg and hypertension as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg (James *et* al., 2014). The measurement of BP was done using a calibrated mercury sphygmomanometer, and the examinations were done after the subjects rested for about 10-15 minutes while sitting with their feet on the floor and arms at the same level of the heart. Obesity was defined as a condition of excess body fat and anthropometrically measured as body mass index (BMI), body weight (kg) relative to body height (m^2). The body weight was measured using a calibrated beam balance and the height using a vertical measuring rod. Obese in this study was defined as BMI ≥ 27 kg/m^2 based on the Riset Kesehatan Dasar (RISKESDAS) 2010 BMI cut-off point of Indonesian adult population (Balitbang Kesehatan, 2010). Furthermore, we measured the waist and hip circumferences using a measuring tape. From the anthropometrical examination results, we also calculated the body fat percentage by using Deurenberg formula for adult (Duerenberg *et* al., 1991).

Sample Collection and Genotyping

Non-fasting patient's blood sample was taken fromthe cubital median vein and mixed into anticoagulant EDTA. Blood plasma obtained by centrifugation was used to measure the leptin concentration using ELISA method. DNA was isolated from the leukocyte by Promega-Madison, Wi, USA (go Taq® green). Polymorphisms genotyping was done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR-RFLP was performed with primers: Forward – 5' -AGG ACC TGA ATT TTG GAC AA-3'; Reverse – 5' -AGG GGC TTC CAA AGT AAA GTG ACA TTT-3'. The PCR program employed denaturation at 94°C for 3 minutes, and then 40 cycles of denaturation at 94°C for 30 seconds continued at 54°C for 30 seconds and 72°C for 30 seconds, and then the last was at 72°C for 10 minutes (Gotoda *et al*., 1997). The PCR product was 78 base pair (bp) and after restricted with 5U AccI enzyme (Genetica Science-Biolabs-New England, Inc), the products were 78 base pair (bp) for lys656 and 47 and 31 bp for Asn656 respectively. The digested products were electrophoresed with 3% Agarose and visualized by ethidium bromide.

Statistical Analysis

In our study, we used SPSS version 20 (IBM Corp.©) to analyze the statistics. Comparisons between the groups and variables, including body weight, waist circumference, hip circumference, the waist-hip ratio were analyzed using independent sample t-test. The other variables such as body mass index (BMI), systole and diastole BP, and body fat percentage, were analyzed by Mann-Whitney test because the data distribution was not normal. The test to know normality of data used was Shapiro-Wilk test. We compared obese and control (non-obese) groups with the occurrence of hypertension using chi-square test. Genotype and allele frequencies of the subjects were analyzed by Chi-square test and Odd ratio. Significancy level was defined as $P < 0.05$.

Table 1. Clinical characteristics of obese and control subjects

Variables	Obese (n = 53)	Control (n = 53)	P-value*
Body weight (kg)	96.7 ± 13.5	61.5 ± 7.0	0.000
Height (cm)	168.3 ± 9.0	166.2 ± 11.2	0.364
BMI (kg/m^2)	34.1± 3.7	22.0± 1.8	0.000
Waist circumference (cm)	104.1± 10.3	75.4 ± 6.0	0.000
Hip circumference (cm)	114.9± 8.4	90.3 ± 6.5	0.352
Waist/hip ratio	0.90± 0.07	0.84 ± 0.08	0.454
Systolic BP (mmHg)	120.2± 8.3	114.7± 8.3	0.055
Diastolic BP (mmHg)	82.1 ± 9.6	77.1± 8.2	0.300
Body fat percentage (%)	32.6± 7.5	17.2± 5.3	0.010
Leptin level (ng/mL)	26.4± 13.1	14.1±8.5	0.000

*Analysed with t-test
BMI = Body Mass Index
BP = Blood pressure

Table 2. Frequency of hypertension in obese and control subjects and frequencies of hypertension in Lys656Asn genotype *LEPR* gene in obese and control subjects

	Hyper tension	Normo tension	P * (OR, CI.95%)	Lys656Asn genotype hypertension	Lys656Asn genotype Normotension	P* (OR, CI.95%)
Obese	19	34	0.026	19	29	0.011
Control	8	45	3.14 (1.13-8.97)	8	45	3.69 (1.31 – 10.66)

*Analysed with Chi Square

RESULTS

Clinical and anthropometrical data of the obese and non-obese subjects were compared as shown in Table 1. The table presented the comparisons of mean ± standard deviation (SD) of the variables between two groups.There were significant differences among two groups in some of the variables, such as body weight, BMI, waist circumference, body fat percentage and leptin level.

In this study, Asn656Asn homozygous genotype was not found either in the obese or the control group. Five (5) persons (9.4%) of obese group were found with Lys656Lys homozygote genotype while none of the subjects in the control group was found with this genotype. Our statistic analysis found that there was significantly different Lys656Asn genotype *LEPR* gene frequency in obese group than control (p = 0.028) (Figure 1).

Table 2 showed the frequency of obese group with higher hypertension and significantly different than the control group (p=0.026). The obese group had 3.14 times risk of hypertension (CI 95%, 1.13-6.97). All of 5 persons with Lys656Lys genotype in the obese group were normotensive. We then compared hypertension frequency of Lys656Asn genotype in obese and control group. This study found a frequency of Lys656Asn genotype in the obese group with hypertension higher and significantly different than the control group. This genotype was also 3.69 times greater a risk factor for hypertension in obese subjects.

With T-test statistic analysis, we found diastolic blood pressure in Lys656Asn genotype of the obese group higher and significantly different with Lys656Lys homozygote genotype (p=0.02). Comparison of Lys656Asn genotype in obese and control group found body weight, BMI, waist circumference, %body fat and leptin level in obese group was significantly higher than the control group (Table 3).

Table 4 showed the phenotype and leptin level in hypertension and normotension of the obese and control group. There were significantly higher differences in body weight, BMI, weight circumference, %body fat and leptin level. This result can be expressed that obesity and leptin level was influenced by hypertension.

Table 3. Relation of Lys656Asn *LEPR* genotype with phenotype, blood pressure, fat mass, and leptin level in obese and control group

Genotype	Lys656Lys Obese (5)	Lys656Asn Obese (48)	Lys656Asn Control (53)	P* Lys656Lys-Lys656Asn obese	P* Lys65Asn Obese-control
Body weight (kg)	98.1 ± 7.6	96.6 ± 14.0	61.5 ± 7.0	0.254	**0.000**
Height (cm)	167.6±7.0	168.3 ± 9.2	166.2 ± 11.2	0.324	0.321
BMI (kg/m^2)	34.98 ± 2.81	34.0± 3.8	22.0± 1.8	0.492	**0.000**
Waist circumference (cm)	102.8 ± 7.0	104.7± 8.6	75.4 ± 6.0	0.197	**0.000**
Hip circumference (cm)	116.4± 5.0	114.7± 8.6	90.0± 6.5	0.247	0.200
Waist/hip ratio	0.88± 0.07	0.91± 0.07	0.84 ± 0.08	0.707	0.448
Systolic BP (mmHg)	119.0± 2.24	120.4± 8.7	114.7± 8.3	0.164	0.154
Diastolic BP (mmHg)	80.00± 0.01	82,4± 10.1	77.1± 8.2	**0.002**	0.105
Body fat percentage	34.74± 8.9	32.3± 7.4	17.2± 5.3	0.417	**0.022**
Leptin level (ng/mL)	26.1± 16.1	26.4± 12.9	14.1 ± 8.5	0.440	**0.001**

*Analysed with t-test
BMI = Body Mass Index
BP = Blood Pressure

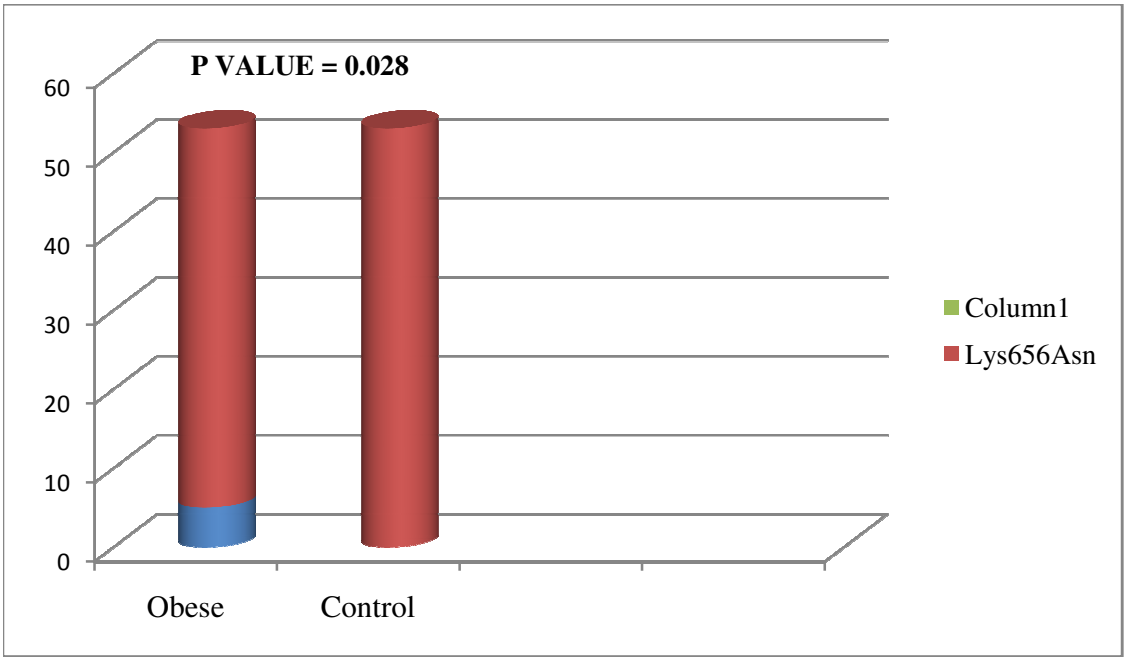

Figure 1. Genotype frequencies of Lys656Asn *LEPR* gene in obese and control groups

DISCUSSION

Our study showed that leptin level in obese subjects was higher than controls and obesity was the risk factor for hypertension. Experiment in animal and human has shown a positive high correlation of adiposity and leptin level. Study by Zheng et al. (2013), found log leptin plasma in hypertension patients higher than controls, and increasing of leptin level not only correlate with hypertension but also correlates with hypertensive left ventricular hypertrophy (LVH). Lu et al.(2000) reported that BMI was a risk factor for hypertension and increase of body weight will increase the blood pressure by 60 – 70%. Hyperleptinemia in obese subjects is caused by leptin resistance, and this condition causes injury, inflammation and metabolic disorders in some organs including the liver, pancreas, and heart (Martin *et al*., 2008).

In this study, Asn656Asn homozygote genotype in obese and control groups was not found. This result may be caused by small number of subject samples or genetic heterogeneity in different ethnic groups. Our study found Lys656Asn polymorphism of LEPR gene as the risk factor for obesity, and obese subjects with Lys655Asn genotype were three times higher as the risk factor for hypertension than control. This result is in agreement with other studies which show that polymorphism of LEPR gene correlates with BMI, body weight and hyperleptinemia (de Oliveira *et* al., 2013). Other studies also confirmed that systolic blood pressure decreased significantly in the Lys656Lys homozygote than heterozygote polymorphism. The difference between this study and other studies are 3.4% mutant genotype (Asn656Asn) in metabolic syndrome subjects ((de Luis *et al*., 2011) and the polymorphism of Lys656Asn was not correlated with metabolic syndrome (Liu *et* al., 2000; de Luis *et* al., 2011; Rosmond *et* al., 2000).

Hypertensive obese subjects in this study were significantly higher in body weight, BMI, waist circumference, % body fat and leptin level compared with control group. This study showed that hypertension may be caused by obesity and leptin level. Some studies correlate obesity with increase of leptin level. Correia and Haynes (2008) explained that most of the obese subjects had leptin resistance and characterized by the increase of sympathetic nerve system activity shown by the rise of catecholamine in the serum to the peripheral sympathetic nervous system in skeleton muscle. Pathogenesis of leptin increase followed by high blood pressure is caused by an increase of epinephrine in urine of a transgenic mouse model. Chronic systemic and intracerebral leptin treatments in the transgenic mouse improve the norepinephrine and cause blocking of α and β-adrenergic receptors and ganglions causing hypertension. Studies in animal and human showed changes in the cardiovascular, neurohormonal and renal functions (Hall *et al*., 2004). The changes were an increase in blood pressure, cardiac output, and heart rate, also activation of the sympathetic nervous system (SNS), renin-angiotensin-aldosterone system (RAAS) and an increase of renal tubular sodium reabsorption. The cause of renal sodium reabsorption increase other than activity SNS and RAAS is physical compression of the kidney by fat accumulation within and around the kidneys. It is attenuated obesity-induced hypertension by at least 50 – 60% (Hall *et al*., 2010). This study can be conducted further with samples that have different gene pool with the Javanese ethnic (western) of Indonesia, such as middle and eastern ethnics of Indonesia and some genetic polymorphism related with hypertension.

CONCLUSION

This study concludes that there was polymorphism of Lys656Asn LEPR gene in obese and control subjects. The Lys656Asn polymorphism of *LEPR* gene is a risk factor for leptin level increase and hypertension.

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