ORIGINAL ARTICLE

Correlation of Leptin Receptor Gene Variation with Blood Pressure and Glucose Level in Type 2 Diabetes Mellitus Subjects of Ternate Population, East of Indonesia

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ABSTRACT

Introduction: The leptin receptor gene (*LEPR*) variation plays an important role in diseases related with obesity which include Type 2 Diabetes Mellitus (T2DM) and hypertension in some populations. The role of this variation is still controversial and not yet studied in the eastern parts of Indonesia. Hence, this study aimed to explore the correlation of leptin receptor variations (Lys109Arg and Gln223Arg) with blood pressure and blood glucose in T2DM in Ternate population. **Methods:** This study examined 136 subjects with the age range of 32-76 years old. Five mL of fasting blood were taken to determine blood glucose levels using the GOD-PAP method, and leukocytes were used for genotyping by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) technique methods. Frequencies of genotypes and alleles were analyzed with chi square tests. Correlations of genotypes with the anthropometric measurements were calculated by logistic regression with significance value if $p<0.05$. **Results:** Variation of Lys109Arg *LEPR* gene did not influence the Body Mass Index (BMI), blood pressure, nor blood glucose level. Variation of Gln223Arg *LEPR* gene also did not influence BMI and blood glucose level, but correlated with blood pressure. Regression analysis after adjusted for age, gender, BMI and blood glucose level showed that this variation remains significantly different. **Conclusion:** Variation of Gln223Arg *LEPR* gene correlated with blood pressure but variation of Lys109Arg *LEPR* gene was not correlated with blood glucose level nor blood pressure. Future study is needed to correlate other genes and examine their effect on metabolic syndrome diseases.

Keywords: Blood glucose, Blood pressure, Gene variation, Leptin receptor

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INTRODUCTION

Circulating leptin plays an important role in regulation of appetite and weight homeostasis and genetic alterations that affect energy metabolism in peripheral tissues can promote increased adiposity and diseases related with obesity (1). Leptin mediates its effects through leptin receptors (*LEPR*), a single transmembrane protein expressed mainly in the central nervous system and other tissues such as pancreatic β cells, liver, skeletal muscle, and adipose tissue (2). The effects of the peripheral hormones derived from adipocytes include regulating insulin secretion and metabolic actions. If leptin deficiency or leptin resistance happens, it is associated with insulin resistance, decreased glucose uptake and oxidation and increased gluconeogenesis (3). Leptin in plasma binds to its receptor to regulate metabolism through Janus kinase 2 (JAK2) in the hypothalamus and peripheral organs forming complexes and activating the phosphatidyl inositol kinase (PI3K) pathway, causing phosphorylated insulin substrate receptors (IRS) and insulin sensitivity to decrease (4). The variations of leptin receptors will interfere with signaling (5), affecting the entering leptin through the blood brain barrier (6). Variation or polymorphism in the encoding gene may be related with its function in the blood affecting the sympathetic nerve system which causes hypertension (7). The human *LEPR* gene is located in 1P31, consisting of 20 exons and 19 introns (8). Genetic variations in the *LEPR* gene cause disruption of *LEPR* expression, failure of leptin action, and the development of obesity with several comorbidities (9). A number of *LEPR* variations have been identified and studied providing different results in both frequency and effect on diseases in the population. Variations Lys109Arg (rs1137100) and Gln223Arg (rs1137101) in *LEPR* genes in several populations correlate with Body Mass Index (BMI), fat mass (10,11), blood pressure (10,12,13), insulin resistance and T2DM (14). Due to the wide distribution of the frequency and inconsistent effects on diseases, the results of these studies in some populations are still

controversial (15, 16).

Ternate is an archipelago in northern Maluku, an area in eastern Indonesia whose mostly low- and middle-income people live. This population is from the Melanesid gene pool which is different from the western of Indonesia, which is mongoloid gene pool. Genetic research in this area is very rare so it is very interesting to study about genetic influences correlated with disease. In this study we examined the effect of variations Gly109Arg and Gln223Arg of the *LEPR* gene as significant risk factors for diabetes and hypertension in obese populations of Ternate.

MATERIALS AND METHODS

A case control study was conducted with volunteer subjects from from UPTD Diabetes Center and Dr. H. Chasan Boesoirie Regional Hospital Ternate island. In total, there were 136 people as subjects consisting of 76 men and 60 women aged between 32 - 76 years. Subjects were divided into two groups: T2DM (72 people) and 64 people as controls without T2DM. Case group was individuals diagnosed with T2DM at the based on criteria from the American Diabetic Association (ADA) 2014 that were fasting glucose levels >126 mg/dL (7.0 mmol / L), or not fasting blood glucose levels >200 mg/dL (11.1 mmol / L), without severe complications. The exclusion criteria in the case group were pregnancy women and undergoing insulin therapy. Controls were individuals who have no history of T2DM or fasting blood glucose levels <126 mg/dL. Exclusion criteria have a family with a history of T2DM. With formula of Sastroasmoro and Ismael (17) with OR was 3.26 (18) and the frequency of Arg223Arg of *LEPR* gene was 0.654, the number of minimal sample = 40. Subjects before taking blood were explained the purpose of the research conducted and asked to sign an informed consent form to participate in this study. Body weight and height were measured by a standardized scale. BMI was calculated by weight (kg) divided by the square of the height (m2). Blood pressure was measured with a calibrated sphygmomanometer. Fasting blood samples (5 mL) were taken from the cubital vein and put in a tube containing EDTA. Blood was separated between plasma and leucocytes. Plasma was used to examine blood glucose level using the GOD-PAP enzymatic method from Dyasis kit. Leucocytes were used for DNA isolation with the Promega kit. Subjects were considered as high blood pressure if they had systolic blood pressure ≥ 140 mmHg and or diastolic blood pressure ≥ 90 mmHg. Subjects diagnosed as T2DM if they had blood glucose levels ≥ 126 mg / dL. All subjects were new T2DM sufferers who had not previously taken anti-diabetic and anti-hypertensive drugs.This research received permission from the Ethics Commission of the Faculty of Medicine, Public Health and Nursing UGM, with the Reference: EC / FK / 0834 / EC / 2017. All protocols and research procedures were in accordance with the principles in the Declaration of Helsinki and subsequent accords.

Genotype examination

Variation of Lys109Arg and Gln223Arg *LEPR* genotypes were examined by PCR-RFLP method.

Variation of Lys109Arg *LEPR* gene

Genotypic examination of Lys109Arg; with the primary used is forward: 5'-TTTCCACTGTTGCTTTCGGA-3'; reverse: 5'-AAACTAAAGAATTTACTGTTGAAACAA-3'. The PCR conditions were as follows: initial denaturation 94 ° C for 3 minutes followed by 40 cycles, 94°C for 30 seconds, 54°C for 30 seconds and at 72°C for 30 seconds using a thermocycler. The 101 bp PCR products were then digested using the restriction of endonuclease HaeIII (New England Biolabs, Beverly, MA) with the fragment results showing 101 base pairs (bp) for AA genotype (Lys/Lys), 70 and 31 bp for GG genotype (Arg/Arg) and 101, 70 and 31 bp for AG genotype (Lys/Arg). After electrophoresis with 3% agarose gel variations were visualized with florosave.

Variation of Gln223Arg *LEPR* gene

The primers used were: forward primer 5'-AAACTCAACGACACTCTCCTT-3' and reverse-primer 5'-TGAACTGACATTAGAGGTGAC-3'. The PCR condition was initial denaturation at 94°C for 3 minutes, 40 cycles at 94°C for 30 seconds, 54°C for 30 seconds, 72°C for 30 seconds, and final extension at 72°C for 4 minutes. The PCR product was 80 bp, then digested with MspI enzyme, giving AA genotype (Gln/Gln) of 80 bp, GG genotype (Arg/Arg) of 58 and 22 bp and AG genotype (Gln/Arg) of 80, 58 and 22 bp (19).

Statistical analysis

Data before processed were analyzed for normality. Data that were normally distributed were analyzed by ANOVA to compare the characteristics of the three study groups. Data that were not normally distributed were presented as median and minimum-maximum and analyzed with non-parametric analysis. Chi-square or Fisher exact tests were used to analyze the frequency of genotypes. Logistic regression analysis adjusted for gender, age, obesity and T2DM was conducted to assess the potential risk of blood glucose and blood pressure levels in genotypic groups. It was stated to be significantly different if $p<0.05$. The program used for statistical analysis was SPSS 17 (IBM, Chicago).

RESULTS

In this study, there were 136 samples consisted of 72 T2DM subjects, 64 controls. The characteristics of the subjects in these two groups had significant differences in body weight, BMI and blood glucose levels ($p <0.05$) (Table I)

Figure 1 compare of Lys109Arg and Gln223Arg *LEPR*

Table I: Characteristics of subjects

Characteristics	T2DM (n=72)	Control (n=64)	*p* value
Age, years old	50.4 ± 8.5	49.4 ± 9.7	0.162
Weight, kg	63.9 ± 7.2	61.8 ± 13.3	<0.001
Height, cm	163.6 ± 5.3	162.9 ± 5.3	0.575
BMI (kg/m^2)	26.6 ± 2.8	23.4 ± 5.3	<0.001
SBP, mmHg	128.9 ± 17.5	123.9 ± 17.5	0.393
DBP, mmHg	79.3 ± 9.9	77.9 ± 9.1	0.796
Glucose, mg/dL	283.6± 79.7	111.8 ± 13.9	<0,001
Hypertension (%)	34.7	18.8	0,037

BMI = Body Mass Index; SBP = Systolic Blood pressure; DBP = Diastolic Blood Pressure

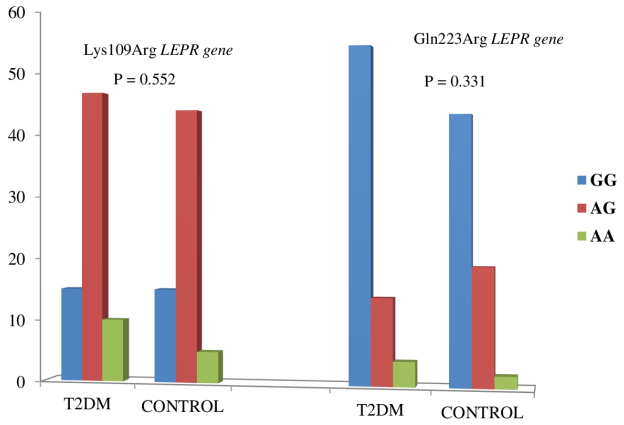

Figure 1: Frequency of Lys109Arg and Gln223Arg *LEPR* gene in T2DM and control group

gene frequency in T2DM and control groups found there were not differences in chi square analysis (P>0.05), then we continued analyzed the genotypes correlated with the characteristic of all subject using regression analysis.

Table II compared the characteristics of all subjects and frequency of hypertension with the variation of Lys109Arg *LEPR* gene. This gene variation did not differ significantly between the genotypes (p>0.05)

Table III showed characteristics of all subjects and frequency of hypertension with the Gln223Ag *LEPR* genotype variation. This table showed the diastolic blood pressure was highest and significantly different in AA genotype compared with GG and AG genotypes. The highest frequency of hypertension was found in AA genotype and significantly different than GG and AG genotypes.

Table II: Characteristics of subjects based on the Lys109Arg LEPR genotype variation

Characteristics	GG (n =30)	AG (n =91)	AA (n = 15)	*p* value
BMI (kg/m^2)	23.77 ± 3.9	24.34 ± 4.2	23.31 ± 4.0	0.496
SBP, mmHg	120 ± 20.8	120 ± 17.1	130 ±22.4	0.571
DBP, mmHg	80 ± 9.8	80 ± 8.7	80 ±13.3	0.592
Glucose, mg/dL	181 ± 85.8	200 ± 114.8	235 ±77.3	0.718
Hypertension (%) Non hypertension (%)	33.3 66.6	25.3 74.7	40 60	0.412

BMI = Body Mass Index; SBP = Systolic Blood pressure; DBP = Diastolic Blood Pressure

Table III: Characteristic of subject based on the Gln223Arg LEPR genotype variation

Characteristics	GG (97)	AG (33)	AA (6)	*p* value
BMI (kg/m^2)	23.34 ± 3.6	24.02 ±5.1	20.98 ± 5.8	0.336
SBP, mmHg	120 ± 16.9	120 ± 15.6	160 ± 34.5	0.066
DBP, mmHg	80 ± 8.9	80 ± 8.9	95 ± 15.5	0.044
Glucose, mg/dL	201 ± 107.4	133 ± 98.5	224 ± 92.6	0.229
Hypertension (%) Non-hypertension (%)	27.8 72.2	21.2 78.8	83.3 16.7	0.008

BMI = Body Mass Index; SBP = Systolic Blood pressure; DBP = Diastolic Blood Pressure.

Because the effect of *LEPR* gene variation in this experiment only found in Gln223Arg *LEPR* gene and different only blood pressure, statistical analysis was continued to correlated this gene variation to the characteristics of subjects with logistic regression with the dependent was blood pressure. This study found, AA genotype was risk factor of hypertension compare the GG and AG genotype. After adjusted with age, gender, BMI and glucose level, this genotype remains as a risk factor for hypertension with Odds Ratio (OR) more than 12 (Table IV).

Table IV. Association of Genetic variant LEPR223 and hypertension

Characteristic	Hypertension	Non-Hypertension	Crude *p* value[a]		Adjusted p value[b]	
			p value	OR (95%CI)	*p* value	OR (95%CI)
GG	27	70		ref		Ref
AG	7	5	0.455	0.698 (0.271-1.78)	0,470	0.699 (0.264-1.849)
AA	5	1	0.011	12.96 (1.45-116.11)	0.027	12.358 (1.332-114.626)
AA/AG	12	27	0.732	1.152 (0.51-2.60)	0.722	1.164 (0.503-2.693)

[a] bivariate analysis; [b] predictive logistic regression adjusted for age, gender, BMI and blood glucose level

DISCUSSION

The results of this study found the carrier of AA Gln223Arg *LEPR* genotype was associated with hypertension but did not correlate with BMI and T2DM. Other studies showed the same results that polymorphism of Gln223Arg was correlated with hypertension (12, 13, 20) and another study showed that the Gln223Arg variation in *LEPR* gene was not associated with obesity in a Turkish children's population (16). This result was different from our previous study with a western Indonesian population, where Gln223Arg *LEPR* gene

correlated with obesity and leptin resistance (21). The frequency of Gln/Gln, Gln/Arg and Arg/Arg found was 0.71, 0.24 and 0.05 in eastern Indonesia study with 0.59, 0.4 and 0.01 in the western Indonesia population. The frequency of variation Gln223Arg *LEPR* gene in several studies shown that there was a relationship with body weight (22-24), elevated leptin levels (20), serum total cholesterol, LDL-C and level of fasting glucose in hypertensive patients in the northern Han Chinese population (25), and with metabolic syndrome (12).

Obese children with Gln223 Gln variation in the *LEPR* gene showed lower post-absorption and postprandial respiratory quotient than Gln223Arg and Arg223Arg genotype (26), while in a Caucasian population Gln223Arg *LEPR* gene was a risk factor for obstructive sleep apnea (4). Carriers of Arg 223Arg in the *LEPR* genotypes have a lower triacylglycerol response than Gln/Arg and Gln /Gln (15). The Heritage Family Study showed a significant relationship between Gln223Arg *LEPR* gene with BMI, skin thickness, fat mass, and lean muscle mass in the Quebec province (27). Another study by Mattevi et al. (28) in the Brazilian population reported there weas a relationship between Gln223Arg *LEPR* gene and BMI, which was stronger in smokers than the general population. Other results which were conflicting with this study found the variation of Gln223Arg *LEPR* gene was not related with hypertension (20,29).

Variation of Lys109Arg in the *LEPR* gene in this study was not correlated with BMI, T2DM and hypertension in the population of Ternate. This result was also different with our previous study with the a population in western Indonesia (Yogyakarta) which found the polymorphism of Lys109Arg was correlated with obesity. The frequencies of Lys/Lys, Lys/Arg and Arg/Arg in this population were 0.22, 0.67 and 0.11 and in Yogyakarta, 0.08, 0.89 and 0.03, respectively (21). The results of this study strengthen the previous research that found the people in eastern Indonesia have a Melanecid genetic profile that is different from western Indonesia which has the genetic signature of the Mongoloid race and the flora and fauna also different in the diversity of plants and animals and the environment which cause a different role in the onset of the disease. This result is in accordance with other studies, showing this genotype variation was not correlated with obesity (11), nor obesity with leptin resistance in the Greek population (25), and did not significantly associate with hypertension (13,20). Other studies show different results, indicating that the Lys109Arg polymorphism was associated with higher systolic blood pressure in men. The combination of gene variations in the 3'flanking region, particularly Lys109Arg and Lys656Arg, were associated with obesity in the population of China (10) and Korea (30) and correlated to waist-hip ratio, 2 hours-oral glucose tolerance test, and HOMA-IR (20), as well as metabolic syndrome (12). The carriers of 109Arg allele *LEPR* gene were associated with high inflammatory condition at a baseline of the study as a marker of obesity which showed significant decrease after treatment with hypocaloric feeding and weight loss. In hypocaloric feeding, Lys109 genotype LPR gene has decreases of body fat mass and cholesterol higher than minor alleles. In animal model found leptin affect vascular phenotypes (31) and correlated with inflammation state (32). Leptin receptor signaling responsible for differences in inflammation and atherosclerosis (33). Su et al. found *LEPR* gene variation (Arg109Lys,Asn656Lys, Gln223Arg and Pro1019Pro) only Pro1019 Pro was correlated with T2DM in systematic review and meta-analysis study (34).

Pathogenesis of leptin causes hypertension indicated by the mechanism of leptin as adipokine hormone, which is bound through its receptors and will transmit the signals through STAT3 in the hypothalamus (35). Changes in receptors due to variations in the encoding gene may be related with its function which will interfere with the apoptosis and cause increased leptin levels and leptin resistance (33). Leptin is an inflammatory agent, and when it increases in the blood affecting the sympathetic nerve system which causes hypertension (7).

Some possible explanations of the differences of these results with others studies are: a) small samples size, b) genetic heterogeneity among ethnic groups in different populations, c) diabetes and hypertension are influenced by many genes, which have complex pathogenesis, where one variation of gene can affect the genes located nearby that influence these diseases, d) the studies are not control all of the confounding factors, and finally, e) differences in criteria exist in determining high blood pressure, or blood glucose levels expressed as diabetes.

CONCLUSION

This study concluded that the variation of Gln223Arg *LEPR* gene is correlated with blood pressure, but not with BMI nor blood sugar levels. Variations of lys109Arg *LEPR* gene were not correlated with BMI, blood glucose nor blood pressure in the Ternate population. This study requires further confirmation to determine other genes correlated to the etiology of a variety of diseases especially metabolic syndrome.

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