Original article

Association between polymorphism of lys198asn endothelin-1 gene and endothelin-1 plasma level in javanese obesity population

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Abstract

Background: Endothelin-1 plays a role as a vasoconstrictor and also inhibits the expression of adiponectin in obesity. The Lys198Asn polymorphism of the endothelin-1 gene will increase the level of endothelin-1 plasma and increase the risk factor of obesity. ***Objective:*** In this study, we investigated the relationship between Lys198Asn polymorphism of endothelin-1 gene and endothelin-1 plasma level among individuals with obesity in Javanese population. ***Method:*** This study was conducted on 61 obese subjects and 65 control subjects. All subjects were measured for anthropometrics and endothelin-1 plasma level by ELISA (Enzyme-linked Immune Sorbent Assay). The polymorphism of Lys198Asn of ET-1 (Endothelin-1) was screened by using PCR-RFLP (Polymerase Chain Reaction- Restriction Fragment Length Polymorphism**).** ***Results:*** TT genotype was a risk factor of obesity (OR 5.344 CI=95% 1.77-16.16) compared with GG genotype. Levels of endothelin-1 plasma were higher in obese subjects than that of control subjects (p=0.013), with TT genotype having the highest endothelin-1 plasma level (p<0.001). ***Conclusion:*** The Lys198Asn polymorphism of endothelin-1 gene plays a role in increasing endothelin-1 plasma levels and is risk factors of obesity.

Keywords: endothelin-1; obesity; polymorphism; vasoconstrictor; ET-1.

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Introduction

Obesity is defined as a state with excessive fat accumulation in adipose tissue inside the body as a result of imbalance between energy intake and activity[1]. Obesity has been a main issue for most countries, and is related with metabolic syndrome. A combination between less physical activity, over eating, and genetic factors may cause overweight and obesity[2]. In 2015, as much as 2.3 billion adults were overweight and 700 million were obese[3].

Endothelin-1 (ET-1) is a peptide consisting of 21 amino acids produced by endothelial cells. The gene involved in coding endothelin-1 is 5.5 kb long located at chromosome 6 and consists of 5 exons and 4 introns and is the strongest vasoconstrictor in human[4]. When first discovered[5], endothelin was a continuation from the previously discovered compound which was endothelium derived relaxing factor. However, studies from porcine thoracic aorta revealed that this peptide binds with its receptor on membrane canals then called endothelin. The increase of endothelin-1 levels has been related with hypertension, stroke, diabetes mellitus[6], and also obesity through inhibition of adiponectin production[7].

Many variants were identified within the endothelin 1 gene. One of them, Lys198Asn polymorphism, is related with increased level of endothelin-1 plasma. This polymorphism is often studied for its relationship with hypertension[8] and stroke, but few studies examined it as a risk factor of obesity. Therefore, it is important to investigate the relationship between this polymorphism and obesity especially in a Javanese population.

Methods

Population and Sample

This study was a case control consisting of 126 Javanese subjects, age 18-35 years old (61 obese subjects and 65 control subjects). Obese subjects were determined using the Asian standard (body mass index (BMI) ≥ 25 kg/m^2) and control subjects

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(BMI 18.5-24.9 kg/m^2). Sampling and reseacrch were held in May 2016 until March 2017.

Biochemical Analyses

Plasma endothelin-1 was measured using ELISA following the protocol provided by Bioassay Tech Laboratory ®.

Detection of Lys198Asn Polymorphism of Endothelin-1 Gene

DNA isolation followed the protocol provided by PROMEGA®. Amplification of endothelin-1 gene using PCR with the forward primer 5'-TCA TGA TCC CAA GCT GAA AGG CTA-3' and reverse primer 5' ACC TTT CTT GGAATG TTT TGAAC-3'. The resulting PCR product was 228 bp. PCR product then was digested by NheI enzyme. The genotype for GG was determined by a single band at 203 bp, GT by 2 bands at 203, 228 bp, and TT by a single band at 228 bp[9].

Statistical Analyses

Data were analyzed with significance level of 5% ($p<0.05$). Normality data were analyzed using the Kolmogorov-Smirnov test. Mean levels of plasma endothelin-1 in the obese and control groups were compared using the Mann-Whitney test. Mean level of plasma endothelin-1 among genotypes was analyzed using the Kruskal Wallis test. Differences between genotype frequencies and allele in each group were analyzed using Chi-Square

Ethical clearance

This study has received approval from the Medical and Health Research Ethics Committee of Faculty of Medicine Universitas Gadjah Mada (reference number KE/FK/532/EC/2016). A hundred and twenty-six subjects involved in this study have signed informed consent forms before filling out questionnaires.

Results

Characteristics of Subjects

This study recruited subjects from students and staff of Medical Faculty of Universitas Gadjah Mada Yogyakarta and Medical Faculty of Universitas Muhammadiyah Purwokerto.

Table 1. Characteristics of obese and control subjects

Variable	Obese n=61	Control n=65	P
Gender:			
- Male(%)	30(49.2%)	36(55.36%)	0.428*
- Female(%)	31(50.8%)	29(44.64%)	
Age (year)	20 (18-35)	20 (18-35)	0.164#
BMI (kg/m^2)	29.90 (25.35-47.18)	21.00 (18.13-24.46)	<0.001#

Characteristic; normality using Kolmogorov-Smirnov:$p<0.05$, data were not normally distributed *using chi-square, #using Mann-Whitney $p<0.05$, significant..

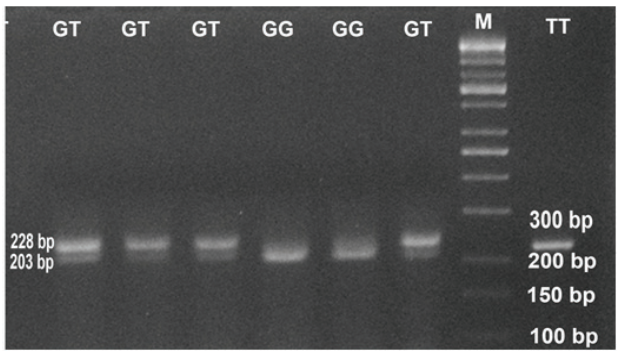
Gender and age did not differ between groups, however BMI was different significantly ($p<0,001$).

Figure 1. Genotyping Endotelin-1 gene.GG is showed at 203 bp, GT at 203,228 bp and TT 228 bp.This genotyping used M (*marker*) from 100 bp,

Table 2. Distribution Genotype and allele of Lys198Asn ET-1 Gene in Obese, Control Groups, and HWE (Hardy-weinberg equilibrium)

Variable	Obese	Control	OR (CI=95%)*	p	HWE		p*
					Observed	Expected	
GG	26(41.5%)	32(49.2%)	0.73(0.326-1.63)	-	58	50	0.01
GT	16(24.6%)	27(41.5%)	5.344(1.77-16.16)	0.442	43	59	
TT	19(31.2%)	6(9.3%)	1.852(1.10-3.11)	0.002	25	17	
T	54	39		0,02			
G	68	91					

*Chi-square, significant if $p<0,05$



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Frequency and Risk Factor of Lys198Asn Genotype in Obese and Control Group

Study of Lys198Asn ET-1 gene showed that frequencies of GG genotype in the obese group (Table 2) was as much as 26 subjects (41.5%), GT 16 subjects (24.6%), and TT 19 subjects (31.2%) (Table 2). Frequencies of genotypes in the control group were GG 32 subjects (49.2%), GT 27 subjects (41.5%), and TT 6 subjects (9.3%). This result is different with some previous studies[10]. Compared to a study by Zhang and Sui[11] in a Chinese population, frequency of GT genotype was as much as 38.6%, which is higher than our result but TT frequency in China was 8.7%, lower than in our Javanese sample population (Table 2). Another study in Kazhakstan[12] showed that GT frequency was 30% in the obese group, which is higher than our Javanese sample population but had lower TT (15%) frequency. These differences may be because of differences in race and area. The result of frequency Lys198Asn genotype of ET-1 gene compare with Hardy-weinberg equilibrium shown there was significantly different (p=0.01) (Table 2). Possibility due to a lack of samples which may have caused this difference between expected and observed outcomes.

The present study showed significant differences in the polymorphism Lys198Asn in the obese group compared with the control group with (p=0.002). The results showed that TT genotype was a risk factor for obesity.

Role of Polymorphism Lys198Asn to Plasma endothelin-1 level in obese and control groups

Table 3. Average ET-1 plasma level between obese and control group

	Obese	Control	P*
ET-1 level (pg/mL)	73,78(20,87-198,78)	44,86(20,03-183,18)	0.013

*Mann-Whitney test, significant if $p<0.05$

Table 3 showed that endothelin-1 level was significant (p=0.013) between the obese group compared to control group. Levels of plasma endothelin-1 in obese and control groups in different genotype were available in Table 4.

Table 4. Levels of endothelin-1 plasma in Lys198Asn ET-1 gene in obese and control groups

	Obese (pg/mL)	Kontrol (pg/mL)	P
GG	45,89 (20,87-86,52)	30,31(20,03-44,86)	
GT	73,78 (50,56-109,46)	69,41(51,69-87,67)	<0,001*
TT	111,45 (104,14-198,78)	163,33 (101,01-183,18)	0,004# 0,004#

*Kruskal-Wallis, significant if p<0.05, #Mann-whitney, significant if p<0.05.

Discussion

After produced from endothelial cell, endothelin-1 is released as paracrine and bound to endothelin receptor A and will continue the signal to activate two pathways (ERK pathway or phosphatase/ kinase pathway). Activated kinase/phosphatase pathway will also activate the cascade which is the calcium canal that will cause vasoconstriction. The kinase/phosphatase pathway also activates DAG (diacylglycerol) which can inhibit the production of adiponectin through the ERK pathway. Adiponectin is a protein that is produced by adipocyte. Adiponectin may manage weight loss, because it can increase GLUT-4 translocation in skeletal muscle according to increases glucose intake and lower glycogen synthesis. When adiponectin was inhibited it will caused insulin resistance, less glucose uptake, etc and it will contributed to obese[13].

Polymorphism of Lys198Asn is exon 5 of pre-pro endothelin-1 gene which causes lysine exchange by asparagine. Mutation in this part of the gene is demonstrated in some studies to elevate endothelin-1 concentration levels[14]. Higher endothelin-1 levels will increase activities in ERK and the kinase/ phosphatase pathways, and subsequent cascades including inhibition of adiponectin[15].

Average level of endothelin-1 plasma (Table 4) among genotypes showed that subjects with TT genotype had higher level of endothelin-1 than GG and GT genotype (p<0.001) whether in obese or in control group. This result is consistent with studies by Jin[16] in Japanese populations and Zang[17], in which subjects with GT and TT genotypes had higher endothelin-1 level along with the polymorphism of Lys198Asn in exon 5 of pre-pro endothelin-1 gene. After pre-pro endothelin-1 then it became pro endothelin-1 after digested by furin-like enzyme and then digested by endothelin converting enzyme (ECE) to become Big ET-1, and then becoming active biologically[18]. Genetic studies show mutations in this gene will affect synthesis of endothelin-1 and increase levels

of endothelin-1 concentration.

Conclusion

The present study showed that TT genotype polymorphism of Lys198Asn ET-1 gene is a risk factor for obesity, and causes increases level of endothelin-1 concentration.

Conflict of interest: None declared

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Authors' Contribution:

Data gathering and idea owner of this study: Dewi Karita

Study design: Dewi Karita, Ahmad Hamim Sadewa

Data gathering:, Ahmad Hamim Sadewa, Pramudji Hastuti

Writing and submitting manuscript: Dewi Karita, Ahmad Hamim Sadewa

Editing and approval of final draft: Dewi Karita, Ahmad Hamim Sadewa, Pramudji Hastuti

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