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Association of CAPN10 SNP-19 (rs3842570) Polymorphism on Fasting Plasma Glucose, Blood Pressure and Body Mass Index of Javanese Type-2 Diabetes Patients

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Abstract. Polymorphism of CAPN10 has been reported to be responsible for risk of Type 2 Diabetes Mellitus (T2DM), and CAPN 10 SNP-19 (rs3842570) is associated with elevation of blood glucose, obesity, and metabolic syndrome. Aims of this study are to analyze the association between polymorphism CAPN10 SNP-19 (rs3842570) on fasting plasma glucose (FPG), blood pressure (BP) and body mass index (BMI) of Javanese T2DM patients. The study sample comprised 107 T2DM subjects as well as 107 healthy subjects of Javanese origin. T2DM subjects were obtained from several Primary Public Health Centers in Semarang. The polymorphism of CAPN10 SNP19 was examined using PCR. FPG, BP, and BMI also were examined. The association between FPG, BP, and BMI in each genotype class was analyzed using Kruskal Wallis test. In the T2DM group, the 2R/3R genotype had the highest mean of FPG ($138,42 \pm 6,77$ mg/dl), BMI ($26,49 \pm 0,41$). However, the highest mean of blood pressure was 2R/2R genotype with systolic $142,00 \pm 7,27$ mmHg and diastolic $83,11 \pm 3,17$ mmHg. There was no significant association between polymorphism in T2DM group with FPG, systolic BP, diastolic BP and BMI with p-value 0,563; 0,734; 0,939; 0,087, respectively. In conclusion, CAPN10 SNP-19 (rs3842570) polymorphism does not contribute to elevating FPG, BP, and BMI of Javanese T2DM patients.

Keywords: CAPN10, polymorphism, Fasting Plasma Glucose, Blood Pressure, Body Mass Index, Diabetes mellitus

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder which is characterized by high blood glucose levels and it was estimated in 2017 there are 451 million people with diabetes worldwide. Based on Basic Health Research (RISKESDA) in 2013, the highest prevalence of diabetes mellitus diagnosed by doctors is in Yogyakarta (2.6%), where most ethnic groups are Javanese [1]. DM becomes the second ranks most frequent in non-communicable diseases after hypertension in Central Java, with its frequency of about 19,22% [2]. Etiological factor of T2DM is caused by interaction between environment and genetic factors. At least there is 40 genes affect to T2DM. Calpain 10 (CAPN 10) gene is one of candidate gene for susceptibility risk factor of T2DM. CAPN 10 gene is affected by



some glucose metabolism, insulin regulation and adipocyte differentiation [3]. A study by Maleki proved that there was an association between T2DM with polymorphism of CAPN10 SNP-43 in the Kurdish ethnic group of West Iran. Otherwise, SNP 19 and 63 did not relate to T2DM [4].

T2DM is characterized by insulin resistance which is considered to be the underlying mechanism of metabolic syndrome. Metabolic syndrome was noted if there is three or more of the following five criteria are met: elevated Fasting Plasma Glucose (FPG), wider waist circumference, lower high-density lipoprotein (HDL), elevated fasting triglyceride (TG) level and, elevated blood pressure [5]. CAPN 10 plays role in evidence of insulin resistance in Spanish population. Research by Saez, 2008 which involved Spanish population, showed that variation of CAPN 10 gene is related with oral tolerance glucose test and Homeostatic Model Assessment of Insulin Resistance (HOMA IR) as insulin resistance indicators. Some genetic studies among the different ethnics showed different results about the relationship between CAPN 10 polymorphisms with parameters such as FPG, elevated blood pressure and BMI. CAPN 10 SNP-19, SNP-43 and SNP-63 did not relate with Body Mass Index (BMI) in Mexican T2DM patients [6]. SNP 43 is an A/G transition within intron-3. SNP 63 is a C/T transition within intron-13. SNP-19 is characterized by two or three repeats of a 32-bp sequence within intron-6. In Japanese population, SNP -19 with haplogenotype 112/121 related with low BMI and low HbA1C [7]. Another study by Ezzidi et al, 2010 that identify BMI among T2DM Tunisian patients found that there was a positive association of genotype 3R/3R with both overweight and obese [8]. A study by Maleki et al., 2014 in Iran proved that in T2DM subject, no significant difference was found in Fasting Plasma Glucose between 2R/2R, 2R/3R and 3R/3R genotype in CAPN10 SNP-19 [4].

Although the variation of CAPN 10 gene has been reported related with T2DM in several populations, relation between SNP 19 with BMI, Fasting Plasma Glucose and blood pressure in Javanese population have not been reported. Javanese are the highest number of tribes in Indonesia. It was found that the majority of Javanese like to consume sweet foods and are processed using coconut milk. This research aims to analyze the association between CAPN10 SNP-19 (rs3842570) polymorphism on BMI, Fasting Plasma Glucose and blood pressure.

2. Materials and Methods

2.1. Materials and Instrumentation

2.1.1. Materials. The whole blood of the subjects who came to the laboratory of primary health centers in the morning after an overnight fast, GeneJET™ Genomic DNA Purification Kit, Ethanol 96%, Hot Start PCR Master Mix Kit, primer forward and reverse CAPN10, 1x buffer TAE, agarose 2%, loading dye, DNA ladder (marker) 100 100 bp.

2.1.2. Instrumentation. PCR amplification used thermal cycler Biorad C1000.

2.2. Methods

This study was observational analysis research with 107 T2DM subjects as well as 107 healthy subjects of Javanese origin. This study used a consecutive sampling method that obtained T2DM patients in several Primary health centers in Semarang that met the inclusion criteria, such as Javanese ethnic of T2DM patients, and aged 30-65 years old. The research was carried out after obtaining ethical approval from the Biomedical Research Ethics Commission of Universitas Negeri Semarang. Informed consent was always given at the beginning of the study to the samples.

2.2.1. Analysis polymorphism of CAPN 10 SNP-19. The blood collection was obtained from peripheral blood, and the DNA extraction was conducted using the GeneJET™ Genomic DNA Purification Kit protocol. The PCR was performed in a final volume of 12,5 uL, containing 4.25 uL of ddH₂O, 6.25 uL PCR master mix, 0.5 uL each of primer and 1 uL of DNA sample. The Primer CAPN10 gene used as the Forward primer was 5'-GTTTGGTTCTCTTCAGCGTGGAG-3' and the reverse

primer was 5'-CATGAACCCTGGCAGGGTCTAAG-3'. The mixture is amplified under the scheme of PCR by initial denaturation at 95°C for 4 minutes and will be completed after 35 cycles each comprising of: 95°C for 1 minute, 60°C for 30 s and 72°C in 1 minute, then 10-minute extension at 72°C. The PCR generated 155 bp DNA products for 2R allele (2 repeats of 32 bp sequence) and 187 bp DNA products for 3R allele (3 repeats of 32 bp sequence) which were visualized in 2% agarose gel.

2.2.2. Measurement of Fasting Plasma Glucose, Blood pressure, and Body Mass Index. Blood pressure and anthropometric measures were measured before the collection of venous blood samples were carried out. Blood pressure was measured using mercury sphygmomanometer with subjects in sitting position. BMI was calculated by dividing the weight (in kilograms) over the height squared (in centimeters). Venous blood samples were collected after T2DM patients fast overnight for measuring Fasting Plasma Glucose.

2.2.3. Statistical Analyses. The mean value of Fasting Plasma Glucose (FPG), Blood Pressure (BP) and Body Mass Index (BMI) in each genotype were presented as means \pm SD. The Kruskal Wallis test was used to compare the value of FPG, BP and BMI between groups with $p \leq 0,05$ were considered as statistically significant.

3. Results

A total sample of 214 individuals who divided into 107 T2DM subjects and 107 healthy subjects shown that in the T2DM subjects, the genotype 2R/3R had the highest mean of FPG (138,42 \pm 6,77 mg/dl), BMI (26,49 \pm 0,41). However, the highest mean of blood pressure was genotype 2R/2R with systolic 142,00 \pm 7,27 mmHg and diastolic 83,11 \pm 3,17 mmHg. In the control subjects, 2R/3R had the highest mean of FPG with 99,53 \pm 2,29 mg/dl, other 2R/2R which had the highest value of BMI (25,10 \pm 0,79), systolic BP (119,42 \pm 3,27 mmHg) and diastolic BP (86,81 \pm 2,59 mmHg) as presented in table 1.

In a table 1 also showed that there was no significant association between polymorphism of CAPN10 SNP-19 (rs3842570) in T2DM subject with FPG, systolic BP (SBP), diastolic BP (DBP) and BMI with p value 0,563 ; 0,734 ; 0,939 ; 0,087 , respectively. No significant results also occurred in the control group for FPG ($p=0,227$), BMI ($p= 0,633$), systolic BP ($p= 0,903$ and diastolic BP ($p=0,543$).

Table 1. Association of Fasting Plasma Glucose (FPG), Blood Pressure (BP) and Body Mass Index (BMI) in each genotype

	T2DM				Not T2DM			
	2R/2R	2R/3R	3R/3R	p	2R/2R	2R/3R	3R/3R	p
BMI (kg/m ²)	24,07 \pm 0,817	26,49 \pm 0,41	25,01 \pm 0,79	0,087	25,10 \pm 0,79	24,54 \pm 0,61	24,15 \pm 0,67	0,633
FPG (mg/dl)	125,67 \pm 9,62	138,42 \pm 6,77	119 \pm 11,68	0,563	93,08 \pm 2,87	99,53 \pm 2,29	94,34 \pm 2,31	0,227
Systolic Blood Pressure (mmHg)	142,00 \pm 7,27	138,36 \pm 2,3	132,57 \pm 4,71	0,734	119,42 \pm 3,27	118,23 \pm 2,37	117,95 \pm 2,40	0,903
Diastolic Blood pressure (mmHg)	83,11 \pm 3,17	82,33 \pm 1,27	81,21 \pm 1,27	0,939	86,81 \pm 2,59	83,13 \pm 1,73	82,98 \pm 1,73	0,543

4. Discussion

Both in T2DM group and control group, the genotype 2R/3R had the highest mean of FPG and there was no significant association between polymorphism of CAPN10 SNP-19 (rs3842570) and FPG. This result similar to the result of another study that proved there was no difference in FPG level among the three genotypes [7]. Another study in Iran by Maleki et al., 2014 proved that in T2DM subject, no significant difference was found in FPG between 2R/2R, 2R/3R and 3R/3R [4]. Mean of FPG in control group among 2R/2R, 2R/3R and 2R/3R genotype in our study is $93,08 \pm 2,87$; $99,53 \pm 2,29$ and $94,34 \pm 2,31$, respectively. Based on a study by Nichols et al, 2011 that investigate the association of normal FPG and the risk for T2DM in Italian showed that FPG between 91 until 99 mg/dl is a strong independent risk for T2DM. It suggests that in our subjects, they are in risk to get T2DM, so it is necessary to identify the subjects to get prevention strategies [9].

T2DM is characterized by insulin resistance which is considered to be the underlying mechanism of metabolic syndrome. Based on the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III), metabolic syndrome was noted if there is three or more of the following five criteria are met : Fasting Plasma Glucose (FPG) over 100 mg/dl, waist circumference over 40 inches (men) or 35 inches (women), fasting high-density lipoprotein (HDL) cholesterol level less than 40 mg/dl (men) or 50 mg/dl (women), fasting triglyceride (TG) level over 150 mg/dl, and, blood pressure over 130/85 mmHg [5]. Diminished insulin sensitivity or insulin resistance is the key of metabolic syndrome and also can act as a cause of hypertension. Insulin acts on nephron of the kidney to stimulate salt reabsorption for maintaining vascular volume which effects on systemic blood pressure. Based on studies proved that stimulation of proximal tubule transport kidney through the insulin or Insulin receptor substrate 2 (IRS2) or phosphoinositide 3-kinase (PI3K) pathway may become the key of hypertension associated with metabolic syndrome [10], [11], [12]. Our results study showed that there was no association between CAPN10 SNP19 in both SBP or DBP. A study investigated another variant of CAPN10 such as SNP43 succeed to examine the effect of variant of CAPN10 as a risk of hypertension in Japanese population [13].

Subject with genotype 2R/3R had the highest mean of BMI in T2DM group, meanwhile, in control group, the the highest mean of BMI was 2R/2R. There was no significant association between polymorphism of CAPN10 SNP-19 (rs3842570) both in T2DM group and control group with BMI. In agreement with the study of Pihlajamaki et al, 2006 which measured intra abdominal fat area of T2DM patients. This study indicates that there is no association between CAPN10 SNP-19 and BMI which correlated strongly with abdominal obesity in TD2M [14]. Another study that investigated polymorphism of CAPN10 SNP-19 in the healthy nonobese patients, found that there was no effect of CAPN10 SNP 19 on BMI and FPG [15]. Study in Colombian population showed that genotype 3R/3R was significantly associated with excess weight more than in normal weight [16]. Our result study was different from a study by Shima et al., 2003 which involved 286 subjects who performed General Health Checkup in Japan that proved subject with genotype 3R/3R had the highest BMI [7]. That study also found that there was a different distribution in BMI among three genotypes (2R/2R, 2R/3R, and 3R/3R). Although the significant differences were only seen between genotype 3R/3R and 2R/3R ($p=0.034$). This finding implies the contribution of SNP19 to the risk of obesity in general Japanese population. Another study by Ezzidi et al that identifies BMI among T2DM Tunisian patients found that there was a positive association of genotype 3R/3R with both overweight and obese [8]. Generally, metabolic syndrome can be summarized into 4 main central features. There are insulin resistance, visceral obesity, dyslipidemia and endothelial dysfunction [5]. Insulin resistance and visceral obesity are absolutely required for metabolic syndrome diagnosis. In an obese individual with insulin resistance, urinary sodium excretion was diminished by insulin, thus insulin ability to stimulate salt absorption was preserved [10]. Therefore, the mechanism of insulin-stimulated salt reabsorption and impaired vasodilation becomes the pathogenesis of hypertension in insulin resistance among obese individual.

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