ACE Gene Polymorphism and Atherosclerotic Lesion of Carotid Artery Among Offsprings of Type 2 Diabetes Mellitus

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ABSTRAK

Tujuan: *untuk melihat hubungan antara polimorfisme gen ACE, konsentrasi serum ACE dan lesi aterosklerotik pada anak kandung subyek DM tipe 2 di Jakarta Indonesia.* ***Metode:*** *studi potong lintang pada 73 anak kandung subyek DM tipe 2 yang tidak memiliki DM dan tidak memiliki hipertensi. Tiap subyek menjalani anamnesis, pemeriskaan fisik dan pemeriksaan laboratorium (tes toleransi glukosa oral, profil lipid, polimorfisme gen ACE, konsentrasi serum ACE) dan pemeriksaan ultrasonografi untuk mencari lesi aterosklerotik.* ***Hasil:*** *proporsi genotip DD, ID dan II gen ACE adalah 10, 38 dan 52%. Terdapat hubungan antara polimorfisme gen ACE dan konsentrasi serum ACE (p=0,000). Di antara 3 genotip, genotip II memiliki konsentrasi ACE serum paling rendah dan perbedaan ini secara statistic bermakna. Prevalensi lesi aterosklerotik pada penelitian ini adalah 45,2%. Tidak terdapat hubungan antara polimorfisme gen ACE dan lesi aterosklerotik.* ***Kesimpulan:*** *insersi dan delesi polimorfisme gen ACE berhubungan dengen konsentrasi ACE serum namun tidak dengan lesi aterosklerotik arteri karotis pada anak kandung subyek DM tipe 2.*

Kata kunci: *polimorfisme gen ACE, konsentrasi serum ACE, lesi aterosklerotik, anak kandung penyandang DM tipe 2.*

ABSTRACT

Aim: *to investigate the association among ACE gene polymorphism, serum ACE level and atherosclerotic lesion in offspring of type 2 diabetes in Jakarta, Indonesia.* ***Methods:*** *a cross sectional study was conducted among 73 nondiabetic and normotensive offspring of type 2 diabetes subjects. Each subject underwent medical history taking, physical examination, laboratory examination (oral glucose tolerance test, lipid profile, ace gene polymorphism, serum ace level) and atherosclerotic lesions (carotid intima media thickness or atherosclerosis plaque) examination using B-mode USG.* ***Results:*** *among 73 subjects, the proportions of genotype DD, ID and II are 10, 38, and 52% respectively. There is association between ACE gene polymorphism and serum ACE level (p=0.000). Among 3 genotypes, II has the lowest value of serum ace level which is statistically significant. The prevalence of atherosclerotic lesion in this study is 45.2%. There is no correlation between ACE gene polymorphism and atherosclerotic lesion.* ***Conclusion:*** *the insertion/deletion (I/D) polymorphism of the ACE gene was associated with serum ACE level but not with atherosclerotic lesion in carotid arteries among offspring of type 2 diabetes subjects.*

Key words: *ACE gene polymorphism, serum ACE level, atherosclerotic lesion, offspring of type 2 diabetes.*

INTRODUCTION

As much as 65% cause of death among diabetes is atherosclerotic cardiovascular disease.[1] Previous studies indicate that endothelial dysfunction plays an important role in the course of atherosclerosis.[2] Atherosclerosis can be determined by measuring the carotid artery intima-media thickness and aortic calcification.[3]

In type 2 diabetes populations, insulin resistance is an important factor in the occurrence of endothelial dysfunction. The impact of insulin resistance are hyperinsulinemia, dislipoproteinemia, hypertension, hirsutism, and prothrombotic states. These conditions might occurred for several years before manifestation of hyperglycemia and was detected as the body's attempts to maintain homeostasis of glucose metabolism.[2]

Activity of the renin-angiotensin-aldosterone (renin-angiotensin system, RAS) plays an important role in the regulation of fluid-electrolyte, blood pressure and kidney function. In type 2 diabetes, there are some abnormalities of RAS systems, including the aldosterone-renin ratio, impaired sensitivity of angiotensin II (AT II) and increased concentrations of angiotensin converting enzyme (ACE).[4] In human, ACE is expressed in endothelial cells throughout the body. Elevated of ACE level and AT II production cause tissue damage through pro-inflammatory and pro-fibrotic effects.[5]

ACE activity was strongly influenced by ACE gene polymorphism. The insertion/deletion of ACE gene polymorphism accounted for 47% of the total phenotypic variance of plasma ACE. Subjects with the DD allele (Deletion/Deletion) showed ACE activity two times higher than in subjects with allele II (insertion/insertion).[6-8] Most recent studies reported that the influence of ACE gene polymorphism on ACE activity affects the outcome of RAS inhibition therapy.[9,10]

A meta-analysis study showed that ACE gene polymorphism significantly has positive association with carotid artery intima-media thickness. This relationship was evident at high-risk populations, both in Caucasian or Asian ethnicity, whereas at general population a positive association was found only in Caucasian.[11-13] Bonet et al. reported an association between increased ACE activity with the risk of glucose intolerance among healthy volunteers.[14]

Offspring of type 2 diabetes is a high-risk population for the occurrence of insulin resistance and or various manifestations of insulin resistance. For example, relatives of type 2 diabetes subjects who are still normoglycaemia have a higher insulin concentration, lower peripheral glucose uptake, and higher accumulation of fat in muscle tissue compared with those without a family history of diabetes.[15,16] Prospective studies showed that there has been established disruption of endothelial vasodilation and carotid artery thickening among offspring of type 2 diabetes who is normotensive and normoglycemia when compared with those without a family history of diabetes.[17,18]

Research on atherosclerosis, the frequency distribution of ACE gene polymorphism, ACE activity and early atherosclerosis in relatives of type 2 diabetes subjects have not been reported. The objective of this study was to investigate the association among ACE gene polymorphism, serum ACE level and atherosclerotic lesion in offspring of type 2 diabetes in Jakarta, Indonesia.

METHODS

A cross sectional study was conducted among 73 nondiabetic normotensive offspring of type 2 diabetes subjects, aged 25-40 years. Each subject obtained informed consent to participate in this study. Subjects were evaluated after an overnight fast and 3 days of abstinence vigorous physical exercise. All the subjects underwent blood pressure and blood glucose examination (using glucometer) before running the study. Subjects were excluded from study if systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or if they were taking antihypertensive medication. They were also excluded if fasting blood glucose examination ≥126 mmHg or they were taking antidiabetic medication. A standard 75 gram oral glucose tolerance test was performed in each subject for evaluating glucose status. Blood pressure was measured in sitting position after the subject is being relaxed for about 15 minutes. The systolic and diastolic blood pressure readings were recorded using a mercury sphygmomanometer with an appropriate cuff size. Waist circumference was evaluated by measuring the abdominal circumference at the level between the last costae and the tip of spina iliaca anterior superior.

Blood samples were taken from the antecubital vein. Blood glucose levels were measured using glucose oxidation method while lipid profile was measured using enzymatic assays. Serum ACE level was measured based on standard sandwich enzyme-linked immune-sorbent assay technology using Boster's human ACE ELISA Kit. ACE gene polymorphism was determined using PCR method.[19] Genomic DNA was extracted using a Genomic DNA Mini Kit (Blood/Cultured Cell) (Geneaid). The ACE gene I/D polymorphism was genotyped by PCR using the forward primer 5'-CTGGAGACCACTCCCATCCTTTCT-3' and reverse primer 5'-GATGTGGCCAT-CACATTCGTC AGAT-3'. A 25 µl of PCR master mix consisting of a final concentration of 10 µM of each primer, Kapa HiFiTM HotStart ready Mix (Kapa Biosystems), and 50 ng genomic DNA was performed. Thermal cycling conditions (GeneAmp® PCR System 9700, Applied Biosystems) were 5 min at 95°C, then 30 cycles consisting of 20 sec at 98°C, 20 sec at 68°C, and 30 sec at 72°C, with a final extension step at 72°C for 5 min. PCR products were separated on a 2% agarose gel, and expected fragment sizes were 190 bp for the D allele and 490 bp for the I allele. Because the D allele in heterozygous samples is preferentially amplified, each sample found to have the DD genotype was subjected to a second, independent PCR amplification with a primer pair that recognizes an insertion-specific sequence (forward primer, 5'-TGGGACCACAGCGCCCGCCACTAC-3' and reverse primer, 5'-TCGCCAGCCCTCCCAT-GCCCATAA-3'), with identical PCR conditions except for annealing temperature of 67°C. The reaction yields a 335-bp amplicon only in the presence of an I allele, and no product in samples homozygous for DD.

Carotid ultrasound examination was done using B mode USG Philips Sonos 5500. Patients were in supine position, neck extension and a light spinning away from the examiner. Transducer placed in the neck, moved from supraclavicular region to the mandibular angle to each side of the right and left. The examination is performed at the Communis Carotid Artery (CCA) sites and bulbus carotid since these locations were the most predilection sites of thickening carotid wall and atherosclerotic plaque. Measurement of the thickened carotid wall and the atherosclerotic plaque was done in the left and right communis carotid arteries and bulbus carotid (each consists of anterior, lateral and posterior side). Assessment of the thickened carotid arteries was reported in milimetres, taken from the average thickness of 3 examined locations (anterior, lateral, posterior) for each of the CCA and bulbus carotid. The proportion of atherosclerotic lesion is reported as the percentage of total subjects who have the atherosclerotic plaque or the thickened carotid arteries.

All statistical analysis were performed using the SPSS 12 statistical software system. Proportion of ACE gene polymorphism was reported as frequency. Variable characteristics were reported as mean (SD) if the distribution was normal and as median (maximum-minimum) if the distribution was not normal. The mean level of serum ACE among three genotype was tested using ANOVA test, while the proportion of aterosclerosis lesion among three genotype was tested using chi-square or fischer test.

RESULTS

There were 73 subjects available in this study, consisting of 26% men and 74% women. All of them were normotensive and had no diabetes. Among them, 69 subjects underwent USG examination. The characteristics of the subjects can be seen in **Table 1**. The total frequency of the D and I alelle were 29 and 71% respectively.

Using Pearson correlation, serum ACE level was strongly associated with the ACE I/D polymorphism ($r=0.55$, $p<0.001$)

Compared with II subjects, serum ACE level was increased in DD and ID subjects by 37% and 42% respectively ($p=0.000$, ANOVA) as shown in **Table 2**.

As shown in **Table 3**, ID genotype has the highest prevalence of atherosclerosis lesion (56%) while DD and II genotypes have a similar value (42.9 and 43.2% respectively). The difference between ID and II genotype in terms of atherosclerotic lesion was not statistically significant ($p=0.592$).

The prevalence of prediabetes (impaired fasting glucose, impaired glucose tolerance or a combination of them) in this study was 26%. Among several risk factors, only males had significant association with atherosclerotic lesion. Other risk factors such as central obesity and prediabetes did not associate

Table 1. Characteristics of subjects

Variables	N(%)	Mean (SD)	Median (min-max)
Sex			
- Men	19 (26)		
- Women	54 (74)		
Age (y)			33 (26–40)
Body mass index (kg/m2)		26.29 (4.60)	
Waist circumference (cm)			83.5 (69–119)
Systolic blood pressure (mmHg)			110 (94–124)
Diastolic blood pressure (mmHg)			78 (60–82)
Fasting blood glucose (mg/dL)		82.60 (8.43)	
2-h post load blood glucose (mg/dL)			107 (63–187)
Total cholesterol (mg/dL)		200.04 (35.94)	
HDL cholesterol (mg/dL)			55 (26–110)
LDL cholesterol (mg/dL)		126.99 (31.50)	
Triglyceride (mg/dL)			84 (32-194)
Serum ACE level (IU/L)		6.02 (2.48)	
Atherosclerosis lesion (%)	45.2		

Table 2. Serum ACE level according genotype of ACE gene

Genotype	Serum ACE level (IU/L)*
DD	7.18 (2.18)
ID	7.79 (2.45)
II	4.51 (1.36)

*mean (SD)

statistically significant although the prevalence of atherosclerotic lesions among those groups showed higher values than those of without central obesity and prediabetes.

DISCUSSION

The proportions of D/D, I/D, and I/I genotype were 10, 38 and 52% respectively. Previous study reported various proportions in ACE gene polymorphism among ethnics.[11] Most of them reported that ID genotype had the biggest proportion, while in Asian populations, DD genotype had the lowest frequency. Those studies have included general populations and high risk populations (hypertensive, coronary artery disease, DM and ischaemic heart disease) but there has been no study with specific population such as offspring of type 2 diabetes. In this study, among offspring of type 2 diabetes, the II genotypes had the biggest frequency and DD genotypes had the lowest one. There are studies in Indonesia that have already reported the proportions of ACE gen polymorphism among diabetes and hypertensive populations. A study by Sinorita in 2010 among 69 type 2 diabetes showed that the proportions of DD, ID and II genotype were 23.19; 18.84 and 57.97% respectively.[20] While a study by Bawazier in 2010 reported that the proportions of ACE gene polymorphism among normo, pre and hypertensive subjects were not different. The II genotype had the highest frequency, followed by ID genotype and the lowest one was DD genotype.[21] This is the first study that reported the ACE gene polymorphism among offspring of type 2 diabetes.

Table 3. Prevalences of atherosclerotic lesions among genotypes of ACE gene*

Genotype	Atherosclerosis lesion (%)
DD	42.9
ID	56
II	43.2

*among 69 subjects

Table 4. Comparison of risk factors proportion based on the presence or absence of atherosclerotic lesion

Risk factors	Atherosclerotic Lesion		p*
	Presence (%)	Absence (%)	
Male sex	68.4	31.6	0.035
Central obesity	55.8	44.2	0.088
Hypertriglyceridemia	48.4	51.6	0.550
Hypocholesterol HDL	52.4	47.6	0.616
Hypercholesterolemia	58.1	41.9	0.124
Hypercholesterol LDL	53.6	46.4	0.430
Prediabetes	61.1	38.9	0.189

*Chi square/ Fischer test

Endothelial dysfunction in high risk groups are influenced by the increased activity of RAS and increase sensitivity of AT II concentration.[4] ACE plays an important role against the two physiological systems, namely the production of angiotensin II and Bradykinin degradation process. Angiotensin II affects cell growth and proliferation by stimulating a variety of cytokines and growth factors that cause endothelial dysfunction by reducing nitric oxide bioavability.[22] Plasma ACE activity is strongly influenced by ACE gene polymorphism. D allele correlated with the higher activity of angiotensin converting enzyme (ACE).

In this study, the ACE polymorphism detected by PCR analysis appears to be associated with serum ACE level. This result supported the previous studies among Caucasian, Australian and Asian that showed ACE gene polymorphism determined plasma ACE activity.[5,12,23,24] Study by Rigat et al among 80 healthy subjects in 1990 reported that ACE gene polymorphism determined serum ACE level and accounted for 47% of the total phenotypic variance of serum ACE. DD genotype had the highest level of serum ACE while II genotype had the lowest one.[5] A study by Larsen in Caucasian population showed that the DD and ID genotypes seem to be associated with a 50% and a 20% increase in plasma ACE activity respectively.[23] Study by Hung among 1111 subjects (general population) in Perth, Australia showed that DD genotype correlated well with the higher plasma ACE activity while II genotype had the lowest plasma ACE activity ($p<0.0001$).[12]

A study by Nakai among 178 patients with CAD and 100 control subjects has reported similar result to Hung. DD genotype had the highest level of plasma ACE.[24] In this study, genotype I/D had the highest plasma ACE activiy and genotype I/I had the lowest one. The differences of plasma ACE activity were statistically significant between II and ID and between II and DD but not between DD and ID. Previous studies supported that alelle D and DD genotype correlated with the higher level plasma ACE activity.[22] This study showed different result which is ID genotype having the highest level of plasma ACE. It may explain that the effect of DD genotype or D alelle on plasma ACE activity was not the same among general population and may be related to ethnicity. The association between ACE gene polymorphism and serum ACE activity has not yet reported in Indonesia.

In this study, we also examined the occurence of early or asymptomatic atherosclerosis among offspring of type 2 diabetes. Previous studies showed that subjects with a family history of DM have multiple cardiovascular risk factors such as higher intra-abdominal fat depth, higher systolic blood pressure, higher plasma concentrations of triglycerides and total cholesterol, lower plasma concentrations of HDL, and lower endothelial dependent vasodilation when compared with subjects without a family history diabetes.[1] Therefore, relatives of type 2 diabetes have a higher risk of atherosclerosis than those of without a family history of DM. Besides that, relatives of type 2 diabetes have higher insulin concentrations compared with those without family history of type 2 diabetes.[15] Study by Balletshofer involving 53 offspring of type 2 diabetes aged around 35 years, using endothelial-dependent vasodilation examination (EDV) and endothelial independent vasodilation (EIV), indicating that they had impaired brachial artery vasodilation although the glucose tolerance was normal and normotensive.[17] Early atherosclerotic lesions can be detected by using non-invasive measurement of intima-media complex thickness (IMT) CCA using ultrasonography (USG).[3] USG examination has a sensitivity of 93,4% and a specificity of 94% to assess atherosclerosis. An increase in the thickness of the carotid artery assessed by USG has been approved by the American Heart Association (AHA) as a diagnostic tool for atherosclerosis and associated with increased risk of cardiovascular events.[3]

Correlation between ACE gene polymorphism and the thickness of carotid artery (early atherosclerosis) in the general population showed controversial results. Studies by Hung in Perth, Australia and by Manami in Japan were unable to get a correlation between ACE gene polymorphism and early atherosclerosis in the general population.[12,13] On the other hand, meta-analysis study by Tabatabei has reported a correlation between ACE gene polymorphism and the thickness of carotid artery.[11] These differences can be influenced by genetic variants of the study population. So far, there has been no report about the association of ACE gene polymorphism with the thickness of carotid artery among relatives of type 2 diabetes. In this study, there is no

correlation between ACE gene polymorphism and atherosclerotic lesion although the proportion of atherosclerotic lesion in ID genotype is higher than that of DD and II genotypes. This is the first study that reported the association between ACE gene polymorphism, serum ACE level and the thickness of carotid artery among offspring of type 2 diabetes. Furthermore, we did not find correlation between traditional risk factors and atherosclerotic lesion, although the prevalence of the atherosclerotic lesion was high enough in this study (45.2%). From several risk factors, male had higher prevalence of atherosclerotic lesion than that of female (p=0.035). While the other factors were not different statistically significant although some risk factors such as central obesity and prediabetes status tended to have higher atherosclerotic lesion than that without central obesity or prediabetes status. It might show that the early atherosclerotic lesions among offspring of type 2 diabetes were not fully aggravated by traditional risk factors such as blood pressure, glucose tolerance, lipid profile and obesity. Other non traditional risk factors such as proinflammatory cytokines also have roles in atherogenic process.[25,26]

In this study, we included group offsprings of type 2 diabetes population aged 25-40 years. The prevalence of atherosclerosis among the general population who underwent general medical check up in China were lower than the prevalences reported in this study (7.2 to 22%).[27] Previous studies in specific populations (autoimmune disease) and high risk populations (pre and post menopause) have also reported lower values of atherosclerosis than that of this study. Study by Mulyasari among 80 women SLE patients reported that prevalence of atherosclerotic lesion was 40%.28 Lucena reported the prevalence of asymptomatic atherosclerosis among peri and post-menopausal women in Brasil was 17.7%.[29] Despite the young age, no hypertension, no diabetes and no or mild lipid abnormality, we found 45.2% atherosclerotic lesions among young, normotensive and non diabetics offspring of type 2 diabetes. The occurrence of atherosclerotic lesion among young adults has been studied before. Strong et al studied the prevalence and extent of atherosclerosis in adolescents and young adults (15-34 years) and reported that atherosclerosis begins in youth, starting with fatty streak accumulation in coronary arteries of the youngest group and these lesions raised rapidly in prevalence and extent through the oldest age group.[30] Since IMT is an early atherosclerotic marker and able to predict future cardiovascular events, this result needs further research to explain and find optimal strategies to prevent cardiovascular complication among this population.[31]

Unfortunately, we did not include a control group in this study, so that we could not see the profile differences between the high risk population (offspring of type 2 diabetes) and normal population (without family history of DM). This study can be the basis for future research about the risk of atherosclerosis among relatives of type 2 diabetes who are still normotensive, non diabetic, and normolipid.

CONCLUSION

The insertion/deletion (I/D) polymorphism of the ACE gene was associated with serum ace level but not with atherosclerotic lesion in carotid arteries among offspring of type 2 diabetes.

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