ABSTRACT

**Aim:** to know the frequencies of insertion/deletion polymorphism in the angiotensin-converting enzyme (ACE) gene among patients with type 2 diabetes and its relationship with metabolic syndrome at Sardjito Hospital Yogyakarta, Indonesia.

**Methods:** we examined 69 patients with type 2 diabetes at Sardjito Hospital Yogyakarta, divided 2 groups based on ATP III criteria of metabolic syndrome. To determine the ACE genotype of the patients, a genomic DNA fragment on intron 16 of the ACE gene was amplified by polymerase chain reaction (PCR) using a forward primer 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and reverse primer 5'-GAT GTG GCC ATC ACA RTC GTC AGA T-3'. II genotype 1 band on 490 bp (homozigot), DD genotype 1 band on 190 bp (homozigot) and ID genotype 2 band (heteroduplex) on 490 bp and 190 bp were separately detected on a 3% agarose gel containing ethidium bromide.

**Results:** of 69 patients with type 2 diabetes, there were 51 females (73.91%) and 18 males (26.09%). Subjects with metabolic syndrome were 49 patients (71.02%) while without metabolic syndrome were 20 patients (28.98%). Subjects with II, DD, ID genotype were 57.97%, 23.19% and 18.84% respectively. The male subjects with II, DD, ID genotype were 55.56%, 27.78% and 16.67% respectively, and the female subject II, DD ID genotype were 58.82%, 21.57% and 19.61% respectively. The association between ACE I/D polymorphism and metabolic syndrome in type 2 diabetes, was not significant (p=0.204).

**Conclusion:** the frequency of ACE I/D polymorphism among type 2 diabetes are 57.97% II, 23.19% DD, 18.84% ID. There is no association between metabolic syndrome and the component of metabolic syndrome and varians of the ACE gene among the type 2 diabetes patients.

**Key words:** insertion/deletion polymorphism of the ACE gene, metabolic syndrome.

INTRODUCTION

The ACE studies in recent years showed as candidate for a variety of diseases. The renin-angiotensin system (RAS) has long been known to be an important regulator of blood pressure and renal electrolyte homeostasis, and this system has also been implicated in the pathological changes of organ damage through modulation of gene expression, growth, fibrosis, and inflammatory response.1-5

A polymorphism in the ACE gene has been described consisting of an insertion or deletion (I/D) of a 287-bp fragment in intron 16. The polymorphism ACE/ID is strongly associated with the level of circulating enzyme.6 This enzyme plays a key role in the production of angiotensin II and in the catabolism of bradykinin, two peptides involved in the modulation of vascular tone and in the proliferation of smooth muscle cells.7 The DD genotype is associated with higher levels of circulating ACE than the ID and II genotypes and studies showed that DD genotype was significantly more frequent in patients with myocardial infarction,8-16 and hypertension.2,17,18

Angiotensin II can also increase insulin sensitivity due to an enhanced blood flow to insulin sensitive tissues19-20 and subject with DD genotype is more insulin sensitive.21-24 Studies about polymorphism ACE/ID has controversial result. Some studies showed no association between the DD genotype and hyperten-sion,10,25,26 other DD genotype studies were associated with an increased susceptibility to type 2 diabetes12,27-29 and dislypidemia.30

There is a controversial result about study in understanding the association between ACE I/D
polymorphism and metabolic syndrome. Some studies in populations also have variation results. A study in Asian subjects showed that II genotype associated with metabolic syndrome, but the other study also conducted in Asian subjects found that the DD genotype associated with metabolic syndrome. So there is a need of further study about the association between ACE I/D polymorphism and metabolic syndrome in Asian subjects.

This study was designed to know the frequencies of insertion/deletion polymorphism in the angiotensin-converting enzyme (ACE) gene among patients with type 2 diabetes and its relationship with metabolic syndrome at Sardjito Hospital Yogyakarta, Indonesia.

METHODS

Sixty-nine patients were studied with type 2 diabetes who consecutively attended the Endocrinology clinic at Sardjito Hospital Yogyakarta, Indonesia between June 2008 and October 2008. The diagnosis of type 2 diabetes was based on the WHO criteria. This study was approved by the human research ethics committee of the hospital, and informed consent was obtained from each patient. All patients underwent complete physical examinations and routine biochemical analyses of blood and urine. The anthropometric parameters data were needed to calculate BMI and waist circumference. Seated blood pressure, plasma biochemical parameters were measured after overnight fasting. Plasma triglycerides, total LDL, HDL cholesterol, and glucose were determined by Beckman Instruments, Galway, Ireland.

Metabolic syndrome was defined according to the proposed criteria released by the ATP III, modified in waist circumference for Asian people. The presence by 3 of 5 factors the basis to establish the diagnosis; these were: abdominal obesity (also highly correlated with insulin resistance) for male waist circumference ≥90 cm and female ≥80 cm, elevated triglyceride of ≥150 mg/dL, reduced HDL-C for male <40 mg/dL and female <50 mg/dL, elevated blood pressure ≥135/80 mmHg, and elevated fasting glucose >110mg/dL (IFG or type 2 diabetes mellitus).

Having been treated with hypertension therapy, subjects who had elevated blood pressure remained in this study, but those who had dyslipidemia before, were given remedies and after having normal blood lipid, were excluded from the study.

Genomic DNA was extracted from peripheral blood with a blood DNA kit (Sepagene Sanyo Junyaku Co., Ltd, Tpkyo Japan). To determine the ACE genotype of the subjects, a genomic DNA fragment on intron 16 of the ACE gene was amplified by polymerase chain reaction (PCR) using a forward primer 5'-CTG GAG ACC ACT CCC ATC TTC CTG-3' and reverse primer 5'-GAT GTG GCC ATC ACA RTC GTC AGA T-3' as described by Rigat et al., 1992. PCR was performed using a standard protocol as follows, with a final volume of 50 μl; 5 μl 10 × PCR buffer, 5 μl 2 mM dNTP, 5 μl forward primer (concentration 20 ng/μl), 5 μl reverse primer (concentration 20 ng/μl), 1.5 μl 50 mM MgCl₂, 0.25 μl Taq polymerase (5 U/μl), 1 μl sample purified genomic DNA (concentration approximately 30 ng/μl), and 5μl dimethyl sulfoxide. Thirty-five cycles of the PCR utilizing a microprocessor-controlled thermal cycler (Perkin-Elmer) were then performed to amplify the desired segment utilizing the following parameters: 94°C for denaturation for 60 seconds, 63°C for 90 seconds for annealing, and 72°C for 90 seconds for extension. The PCR products were subjected to electrophoresis on 1.2% agarose gels and the nucleotide bands visualized by ethidium bromide fluorescence and photography. The deletion polymorphism is characterized by a 190-bp fragment, whereas the presence of the insertion leads to a 490-bp fragment. Heterozygotes exhibit an intermediate band which is most likely a heteroduplex DNA fragment.

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences 15 (SPSS 15). Data are presented as mean ± SD. The statistical difference in distribution of ACE genotypes with metabolic syndrome and the components were analyzed with Kolmogorov Smirnov.

RESULTS

Of 69 patients with type 2 diabetes, there were 51 females (73.91%) and 18 males (26.09%). Subjects with metabolic syndrome were 49 (71.02%) and without metabolic syndrome were 20 (28.98%). The I allele were 67.39% and the D allele were 32.61%. Subjects with II, DD, ID genotype were 57.97%, 23.19% and 18.84% respectively. The male subjects with II, DD, ID genotype were 55.56%, 27.78% and 16.67% respectively, and the female subject II, DD ID genotype were 58.82%, 21.57% and 19.61% respectively.

The association between ACE I/D genotype and metabolic syndrome and its variables in type 2 diabetic patients with Kolmogorov Smirnov analysis were not significant (p>0.005).
This study showed that D allele was less frequent than I allele (32.61% vs 67.39%) and the DD genotype was less frequent than II genotype (23.19% vs 57.97%). Studied in Asian subjects showed the DD genotype was significantly less frequent than in non-Asian subjects.24,31,36

In this study a group of metabolic syndrome subjects were compared with a group of non metabolic syndrome subjects in type 2 diabetic patients. The ACE I/D genotype distribution was not associated, and when we analyzed component of metabolic syndrome such as central obesity, hypertension, and dyslipidemia, the distribution of ACE I/D genotype was also not associated.

The results of this study were different from the study conducted in Chinese patients with type 2 diabetes. This study showed that, in type 2 diabetes patients DD genotype was also less frequent than II genotype (11.1% vs 45.7% vs),31 but patients with metabolic syndrome were more often carriers of the D allele (DD/ID) than the patients without metabolic syndrome. A study in Hongkong performed in Chinese populations with metabolic syndrome subjects found that DD genotype also less frequent than II genotype (6.7% vs 47%), but the result of this study showed a higher frequency of I allele containing genotypes in metabolic syndrome groups.24 Study in Caucasian found that the DD genotype were more frequent than II genotype but there were no association between ACE gene I/D and metabolic syndrome.37,38

Insulin resistance is a potential etiology of the metabolic syndrome. Association of insulin resistance and the ACE I/D polymorphism have been reported by a number of studies, but showed controversial results. Several studies reported the association between insulin resistance and the ACE I/D polymorphism,21-24,31,40 but some others reported no association.12,13,27,37,39

The DD genotype is associated with higher levels of circulating ACE.6,8 This enzyme plays a key role in the production of angiotensin II and in the catabolism of bradykinin.7 The influence of angiotensin II in occuring hypertension also plays a role in resistance insulin via increase insulin sensitivity due to an enhanced blood flow to insulin sensitive tissues.19-20 The limitation of this study and also other studies 24,31,37,38 about the association between ACE I/D polymorphism and metabolic syndrome, was that the authors did not measure ACE plasma level and so then, did not know the ACE plasma activity in metabolic syndrome. The association between metabolic syndrome and the ACE I/D polymorphism remains to need further studies.

CONCLUSION

The frequencies of ACE I/D polymorphism among type 2 diabetes are 57.97% II, 23.19% DD, 18.84% ID. There is no association between metabolic syndrome and the component of metabolic syndrome and varians of the ACE gene among the type 2 diabetes patients.
REFERENCES


