ANGIOTENSIN-CONVERTING ENZYME AND ITS GENE POLYMORPHISM (I/D) IN ESSENTIAL ARTERIAL HYPERTENSION

Mohamed A. Taher,[*a] Ahmed M. Boghdady,[b] Eman A. Sabet,[b] Usama A. Arafa[b] and Ahmed I. Abd Elneam[c,d]

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The polymorphisms of ACE gene is investigated using polymerase chain reaction for detection of ACE I/D genotype frequency in hypertensive cases in sector of Egyptian population. Serum ACE is significant higher in hypertensive group than normotensive one (27.5± 4.4 and 21.3± 5.4 ng mL^{-1}, respectively. Mean SBP (systolic blood pressure), DBP (diastolic blood pressure) and urinary protein excretion in DD genotype were 181±17 mmHg, 112±19 mmHg, 3.8±0.48 mg mL^{-1} and 141.5± 36.8 mg day^{-1}, respectively but in II genotype these were 142± 10 mmHg, 99± 13 mmHg, 4.35±0.4 mg mL^{-1} and 116.4± 36 mg day^{-1}, respectively. In hypertensive patients, serum ACE levels in DD, ID and II genotypes were 36.2± 5.7, 22.1± 3.7 and 24.5± 4.9 unit ?, respectively. D/D ACE gene polymorphism plays a role in the development of target organ damage in severe essential hypertension and might have a prognostic value. It can be considered as one of the risk factor of hypertension.

*Corresponding Authors
Tel: 002- 01226388153
E-Mail: abdelmoaty5@yahoo.com
[a] Medical Biochemistry department Sohag Faculty of Medicine, Sohag University Egypt.
[b] Internal Medicine Departments, Sohag Faculty of Medicine, Sohag University Egypt.
[c] Molecular Genetics and Enzymology Department, Human Genetic Division, National research Center, Cairo, Egypt.
[d] Medical Biochemistry, Department Faculty of Medicine Al Dawadme Shaqra University.

Introduction

Angiotensin-converting enzyme (*EC 3.4.15.1 or ACE*) is Zinc-Metallopeptidase.[1] ACE is secreted in the lungs and kidneys by cells in the endothelium (inner layer) of blood vessels.[2] It indirectly increases blood pressure by causing blood vessels to constrict by converting angiotensin I to angiotensin II which constricts the vessels and stimulates aldosterone secretion and inactivating bradykinin which is a potent vasodilator.[3] As It is a part of RAAS (renin angiotensin aldosterone system), it also, converts Aβ42 (which aggregates into plaques) to Aβ40 (which is thought to be less toxic) forms of beta amyloid. So, it may have a role in preventing accumulation of Aβ42 and progression of dementia.[4] For these reasons, ACE is also known by the following names: *dipeptidyl carboxypeptidase I, peptidase P, peptidyl dipeptide hydrolase, endothelial peptidyl dipeptidase, kininase II, carboxycathepsin, hypertensin converting enzyme* and *dipeptide hydrolase.*[5-7]

ACE gene is located at Location: 17q23.3 and is 21-kilo bases (kb) long and comprises 26 exons and 25 introns.[8] I/D polymorphism was leading to the presence (I) or absence (D) of 287-bp sequence of DNA in intron 16 of the ACE gene as an alu repeat sequences.[9] I/D polymorphism of ACE gene is not only limited to tissues but it is also detected in tissue ACE levels.[10] No evidence was found to support linkage between the ACE locus and essential hypertension.[11] Likewise, another study failed to find a significant association between the I/D polymorphism and blood pressure status in subjects with high or low blood pressure and in their offspring.[12] This lack of association was reported in later studies.[13-17] However, several other studies reported a positive association between the D allele and high blood pressure.[18-20]

Therefore, we carried out this study to determine if this ACE I/D polymorphism is associated with an altered risk of hypertension and its relation to serum ACE in our population.

Patients and methods

Study subject

Sixty-eight normotensive subjects and sixty six hypertensive patients with SBP ≥ 140 mmHg or DBP ≥ 90 mmHg (WHO/ISH 2003)[21] were selected without history or sign of any other cardiac diseases. All subjects and patients were recruited from Internal Medicine outpatient clinic of Sohag faculty of Medicine from June 2015 to August 2015. Complete personal history from each subject and echocardiography for each hypertensive patients was studied to know the severity of aortic valve calcification. 10 ml blood samples were collected on Na_2EDTA to detect ACE I/D polymorphism. Another blood sample was taken, centrifuged and stored at -20 °C until use. Collected serums were used to assay cholesterol, triglyceride, LDL, HDL, urea and creatinine and ACE. Urinary protein excretion (g day^{-1}) and 24-hour creatinine clearance (mL min^{-1}) were investigated.

The Ethics Committee at Sohag University approved this study protocol and written consents were obtained from all patients.

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Serum ACE detection

Serum ACE concentrations were measured using Human ACE Quantikine ELISA Kit from R&D Biotechne. This assay used the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human ACE is pre-coated onto a microplate. Standards and samples are pipetted into the wells and the immobilized antibody binds any ACE present. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human ACE is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and yellow color develops in proportion to the amount of ACE bound in the initial step. The color development is stopped and the intensity of the color is measured by using microplate reader at 450 nm.

Molecular studies

DNA extraction

Genomic DNA was purified from whole blood with the QIAamp® DNA BloodMini Kit according to the Blood and Body Fluid Spin Protocol in the accompanying handbook. DNA was eluted in 200 µl elution buffer and stored at – 20°C.

Detection of ACE I/D polymorphism

To determine the ACE gene I/D polymorphism, a genomic DNA fragment on intron 16 of the ACE gene was amplified by using Polymerase Chain Reaction (PCR) method with a pair of oligonucleotide primers: The upstream of primer sequence was: 5'-CTG GAG ACC ACT CCC ATC CTT TCT -3' and the downstream was: 5'- GAT GTG GCC ATC ACA TTC GTC AGA T-3'. The PCR amplification products were obtained using 25 µL reaction system containing; 50 ng μL^{-1} genomic DNA, double distilled H_2O 15.7 µL, 10xbuffer 2.5 µL, dNTP (10 mmol L^{-1}) 0.5 µL, each of primer sequences (10 µmol L^{-1}) 0.5 µL, $MgCl_2$ (25 mmol L^{-1}) 2 µL and 1U Taq enzyme on a PTC-200 thermal cycler (MJ Research Company).

After an initial denaturation at 94 °C for 5 min, the DNA was amplified by 35 PCR cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min and elongation at 72 °C for 1 min, followed by a final elongation at 72 °C for 10 min. PCR products were separated and sized by electrophoresis on a 2 % agarose gel containing ethidium bromide.

Statistical analysis

Data was presented by means ± SD (standard deviation) and percentages.The compiled data were computerized and analyzed by SPSS version 12. The following tests of significance were used: Analysis of variance (ANOVA) test between more than two means, t-test between mean we used analyze mean difference, *t*-test between percentage to analyze percent difference and chi–square. A level of significance with $p \leq 0.001$ was considered highly significant and $p > 0.05$ was considered insignificant.

Results

A total of 66 hypertensive patients and 68 control subjects were included in this study. 55 males and 11 females (M/F ratio=5) were included in hypertension group but normotensive subjects consisted of 42 males and 26 females (M/F ratio = 1.6).

General and clinical characteristics of all the subjects enrolled in this study are shown in table (1). High serum triglyceride, LDL and ACE with low HDL in hypertensive patients than control subjects were observed. In addition, there was significant 24 h proteinuria in hypertension than control group.

Table 1. General and clinical characteristics of normtension and hypertension groups.

Variable	Normotensive subjects (mean ± SD)	Hypertensive patients (mean ± SD)
Age (years)	45 ± 17	42±14
Mean BMI (kg m^{-2})	31.9± 6.4	32.4 ± 9.3
Total cholesterol (g L^{-1})	2.1± 0.46	2.1± 0.48
LDL(g L^{-1})	1.2± 0.2	1.4± 0.3*
HDL (g L^{-1})	0.49± 0.06	0.42± 0.04*
Triglyceride (g L^{-1})	1.74± 0.43	2.10± 0.55*
Urinary protein excretion (mg day^{-1})	88.5± 24.4	122.4± 36.6*
Creatinine clearance (mg day^{-1})	145.3± 15.2	146.8± 18.7
Serum ACE (ng mL^{-1})	21.3± 5.4	27.5± 4.2*

*: Significant change ($p< 0.05$) between normotensive subjects and hypertensive patients

Table 2. Blood pressure, serum lipogram, urinary protein excretion and creatinine clearance in different ACE genotypes:-

Parameter	DD (mean ± SD)	ID (mean ± SD)	II (mean ± SD)
SBP (mmHg)	181± 17	132± 11*	142± 10$^{\infty}$
DBP(mmHg)	112± 19	94± 16*	99± 13$^{\infty}$
Total cholesterol (g L^{-1})	2.06± 0.48	2.1± 0.49	2.1± 0.49
LDL(g L^{-1})	1.36± 0.31	1.36± 0.3	1.42± 0.3
HDL (g L^{-1})	0.38± 0.05	0.43± 0.04*	0.44± 0.04$^{\infty}$
Triglyceride (g L^{-1})	2.13± 0.06	2.09± 0.5	2.08± 0.55
Urinary protein excretion (mg day^{-1})	141.5± 36.8	110.3± 36.1*	116.4± 36$^{\infty}$
Creatinine clea-rance (mg day^{-1})	149.6± 19.2	145.7± 18.5	145.1± 18.4

LDL: Low-density lipoprotein, HDL; High-density lipoprotein; SBP systolic BP; DBP diastolic BP. *: Significant change ($p< 0.05$) between ACE ID and DD. ∞ : Significant change ($p< 0.05$)between ACE II and DD.

The specific segment of ACE gene was amplified into 190 bp amplicon in case of homozygous DD genotype, 490 bp in case of homozygous II genotype and both in case of heterozygous DI genotype (Fig. 1). In the current study, there were significant association between severe hypertension (SBP≥ 180 or DBP≥110 mmHg) and ACE DD

genotype. Furthermore, there were significant association between low serum HDL, microalbuminuria (30- 300mg/d) and ACE DD genotype (Table 2).

Table 3. Relation between serum ACE and its genotypes in normotensive Subjects and hypertensive patients.

GENATYPES	Serum ACE (ng mL^{-1})	
	Normotensive subjects	Hypertensive patients
DD	21.3± 4.1	36.2± 5.7*
ID	20.4± 4.2	22.1± 3.7©
II	22.2± 4.9	24.5 ± 4.9∞

*: Significant change between serum ACE in ACE DD genotype in hypertensive patients and that in normotensive subjects.

©: Significant change between serum ACE in DD genotype and that ID in hypertensive patients.

∞: Significant change between serum ACE in DD genotype and that II in hypertensive patients.

In the present study, significant high serum ACE levels were associated with ACE DD genotype in hypertension that controls. In addition, serum ACE levels increased in ACE DD genotype than II or ID genotype in hypertensive patients (Table 3).

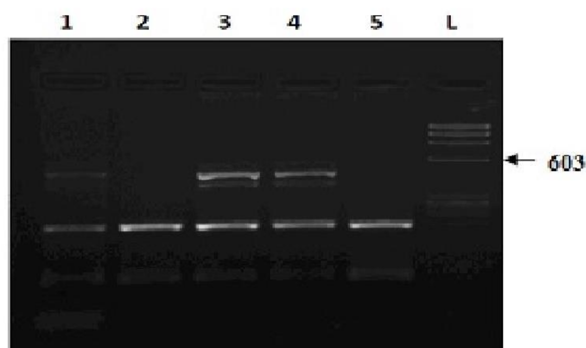

Figure 1. PCR amplification for ACE gene for some patients with ACE polymorphism. Lanes 1, 3 and 4 ACE I/D; Lanes 2 and 5 ACE D/D polymorphism.

Discussion

The renin-angiotensin-aldosterone system (RAAS) is an important system in regulating blood pressure and electrolyte balance. RAAS gene polymorphisms have been extensively studied to determine the genetic susceptibility to hypertension.[1] It also may play an important role in the degree of hypertension.

Accordingly, it was shown that plasma renin activity is an important and independent risk factor for cardiovascular complications in a barge number of patients with essential hypertension.[22] In our results, serum ACE was higher in hypertension than non-hypertensive subjects especially in severe hypertension (SBP ≥ 180, or DBP ≥ 110) where, it increased about two times than that in non-hypertensive subjects. In addition, there were a positive correlation between serum ACE and D/D homozygous ACE gene polymorphism. Our results were agree with Ay[23] and Cardoso,[24] who found that DD genotype is associated with higher plasma ACE activity.

An increased plasma and serum ACE level is genetically determined by D/D polymorphism of the ACE gene. The D/D genotype is associated with higher ACE expression and activity and therefore might predispose individuals to hypertension.[20] Several studies of the association between DD genotype and hypertension showed conflicting results. There was no evidence to support linkage between the ACE locus and essential hypertension.[11] Several authors also did not find a significant association between I/D polymorphism and blood pressure status in subjects with high or low blood pressure and in their offspring.[12-17] Agachan,[18] Kario,[19] and Giner,[20] reported a positive association between the D allele and high blood pressure. This study reports that DD genotype and the D allele of the ACE gene are strongly associated with hypertension (specially severe hypertension) compared to healthy individuals, and they confer increased risk of hypertension.

The observed controversies may be due to ethnic and geographic differences. A study comparing Caucasians and Afro-Caribbeans showed that DD polymorphism is associated with essential hypertension in Afro-Caribbean but not in European descent.[25] Turkish population is found to have no significant association between ACE gene polymorphism and hypertension.[26] The ACE D allele is associated with higher serum ACE levels and increased conversion of angiotensin I to angiotensin II in white populations.[18] Previous studies suggest that ethnicity modulates the relationship between the ACE I/D genotype and serum ACE activity. For example, Bloem and colleagues (1996) confirmed the relationship between the number of ACE D alleles and increasing serum ACE activity in whites but found no correlation between the ACE I/D genotype and serum ACE in blacks.[27] In contrast, Forrester and colleagues 1997 reported a relationship between the ACE I/D polymorphism and serum ACE in individuals of African descent, similar to that previously reported in whites.[28]

The increased risk of hypertension with the D/D genotype may be explained by catalysis the conversion of angiotensin-I to the angiotensin II and inactivation of bradykinin, a potent vasodilator.[3] Also, angiotensin is a pro-inflammatory and pro-oxidant, thus causing cellular toxicity and apoptosis and studies have demonstrated that chronic low grade systemic inflammation can predict the future risk of hypertension (2). In the present study, the D/D genotype is not only a risk factor for hypertension but it is also associated with severe grade of hypertension. In addition, D/D genotype is associated with low serum HDL level that can explain its relation to the grade of hypertension. Cardoso reported that D allele might increase the risk of cardiovascular disease by facilitating the development of left ventricular hypertension and low HDL cholesterol, especially among men.[24]

In the present study, the D/D genotype is associated with the present of microalbuminuria in hypertensive patients. Redon and colleagues, 2000 found that the D allele in hypertensive patients poses a higher risk for

microalbuminuria and treatment with ACE inhibitors produces a greater reduction in microalbuminuria in hypertensive patients.[29]

In conclusion increased activity of the ACE (renin-angiotensin- aldosterone system) secondary to its D/D gene polymorphism might play a role in the development of target organ damage in severe essential hypertension and might have a prognostic value.

Although our study sample was relatively small as compared with other epidemiological and association studies, the result of this study supports the hypothesis that the increased activity of the ACE (renin-angiotensin-aldosterone system) secondary to its D/D gene polymorphism might play a role in the development of target organ damage in severe essential hypertension and might have a prognostic value and considered as one of risk factor of hypertension. In addition, ACE polymorphism plays an important role in the pathogenesis of hypertension. However, further studies with larger sample size are necessary to confirm the association of I/D polymorphism of the ACE gene and hypertension. In addition, further investigation is needed to understand the possible role of other polymorphisms of RAAS genes in relation to essential hypertension in Egyptians.

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