ORIGINAL ARTICLE

The Relationship between Different Polymorphisms of ACE Gene with Hypertension

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ABSTRACT

Background: Hypertension is an important risk factor for a variety of cardiovascular, renal and neurological diseases. Given the high prevalence of hypertension in the country, its complications and problems, and the necessity of identifying the risk factors for hypertension
Aim: To investigate the association between different ACE gene polymorphisms and hypertension.
Methods: This cross-sectional study was performed on patients with primary hypertension who referred to Heart Clinic and Emergency Department of Imam Khomeini Hospital in Ahvaz in 2018. Fourty patients with hypertension were compared with 32 healthy individuals. The addressed factors included age, gender, BMI, systolic and diastolic blood pressure, ACE gene polymorphism and medications.
Results: In this study, which was performed on 40 patients with hypertension and 32 healthy individuals, the mean ages of the case and control groups were 50±8.54 and 45.5±7.54 respectively. The BMI in the case group was higher than the control group and there was a statistically significant relationship between BMI and the two groups. The results showed that the mean of SBP and DBP in the case group was much higher than the control group and a significant relationship was found between SBP and DBP in the two groups. Using the independent t-test, a significant difference was observed between the mean age, BMI, SBP and DBP and hypertension of the case and the control groups ($P < 0.05$).
Conclusions: The present study showed that primaryhypertension is associated with the ACE polymorphisms.
Keywords: Hypertension, Polymorphism, ACE Gene

INTRODUCTION

Hypertension is a public health problem that has affected a large population around the world[1,2]. Hypertension is one of the risk factors for cardiovascular disorders and a major cause of morbidity and mortality around the world, which is characterized by high blood pressure (systolic blood pressure (SBP≥140 mmHg and / or diastolic blood pressure (DBP ≥90mmHg)[3].

This complex and multifactorial complication may lead to complications such as stroke, heart disease, kidney failure, blindness, and cognitive impairment. Hypertension is one of the most important and correctable risk factors for premature death in the world and it is directly associated with death from stroke, coronary heart disease, myocardial infarction etc. About 9.4 million deaths from hypertension are reported worldwide each year[4,5].

Numerous genetic, environmental, and demographic factors are involved in the development of essential hypertension[6, 7]. Genetic factors are responsible for about 30-60% of blood pressure cases, and in other cases, cultural factors such as stress, diet and physical activity appear[6].

Genetic susceptibility increases sensitivity to complex disorders and various risk factors including high blood pressure[8,9]. Each person's genetic makeup is an important risk factor for hypertension susceptibility. So far, about 2,129 blood pressure-related genes have been identified[10].

Blood pressure is mostly regulated by the renin-angiotensin-aldosterone (RAAS) system, which is key role in regulating electrolyte balance as well. Therefore, many studies have been performed on RAAS genetic polymorphism to determine the genetic susceptibility to hypertension[6].

The angiotensin converting enzyme (ACE) gene is one of the main genes in RAAS and the gene is considered for the study of hypertension[11]. The ACE enzyme is a metalloproteinase (dipeptidylcarboxypeptidase) that converts inactive angiotensin I to active angiotensin II, which is a potent vasoconstrictor[12]. Angiotensin II causes vasoconstriction and aldosterone secretion. Thus, ACE plays an important role in regulating the blood pressure. ACE protease activates bradykinin (a potent vasodilator). Therefore, increased serum ACE activity is involved in increased blood pressure or hypertension (6). The ACE gene consists of 1306 amino acid sequences. Additional polymorphisms (I) and deletion (D) of the ACE gene are one of the well-known polymorphisms in RAAS[6]. The ACE (I/D) (insertion/deletion) is a 287-base pair Alu located on Intron 16 on chromosome 17. Because of its location in the non-encoded region, the ACE (I/D) gene polymorphism regulates the ACE serum activity

Some studies have shown the association between the ACE (I/D) gene polymorphism and cardiovascular complications[12-15]. However, the relationship between the I/D polymorphism and blood pressure is still unclear. Some studies have shown an association between ACE I / D polymorphism and essential hypertension in which the DD genotype has been associated with increased blood pressure in different populations[3,6,18-18]. However, other studies have failed to find any relationship[19,20] .

The ACE I/D polymorphism is associated with increased ACE levels in plasma, which increase angiotensin II levels (a key factor in increasing peripheral resistance) in plasma. The mean ACE plasma level in DD cases is about twice that of cases II, and ID cases have moderate levels (21 and 22). Although I/D polymorphism plays an insignificant role in the ACE gene, higher levels of

allele -D-linked ACE may lead to more angiotensin II in the cardiovascular tissue, which predisposes cardiac injury[6].
Due to the high prevalence of hypertension in the country, its complications and problems, and the necessity of identifying the risk factors for hypertension, this study is conducted to investigate the association between different ACE gene polymorphisms and hypertension.

MATERIALS AND METHODS

This case-control study was performed cross-sectionally on patients with primary hypertension who referred to Heart Clinic and Emergency Department of Imam Khomeini Hospital in Ahvaz in 2018. A group of healthy and volunteer individuals was considered as control. This group included healthy individuals with no history of underlying diseases (diabetes, hypertension, and hyperlipidemia) or heart problems that were voluntarily selected from hospital staff and patient companions. The patients with blood pressure were also divided into controlled and uncontrolled blood pressure classes.

All eligible individuals enrolled according to the inclusion and exclusion criteria and after providing the necessary explanations about the purpose and manner of implementation of the project after providing their informed and written consent.
The inclusion criteria:

- Tendency to participate in the study
- 30 to 65 years of age
- Lack of known heart disease

The exclusion criteria:

- Patients with renal, hepatic and cardiac abnormalities
- History of heart attack or cerebrovascular problems such as a history of stroke
- Patients with secondary hypertension

2.5. Grouping the subjects
In this study, the subjects were classified in different groups based on JNC-7 criteria (23) (case and control groups were matched in terms of age and gender):

1. Control group: healthy people with no history of blood pressure and other underlying diseases
2. Case group: People with primary blood pressure diagnosis (controlled and uncontrolled)
   - Normal blood pressure: SBP <120 mmHg and DBP <80 mmHg
   - Pre-hypertensive: SBP = 120–139 mmHg or DBP = 80–89 mmHg
   - Hypertensive: SBP≥140 mmHg or DBP ≥90 mmHg (hypertensive without medication - hypertensive with medication)

   People with normal blood pressure are considered as controls.

Evaluation of research samples: The demographic (age, genderand race), somatometric (including height, weight, body mass index (BMI), waist circumference (WC), waist to hip circumference ratio (WHR)), lifestyle (education, alcohol consumption, smoking) , family history of blood pressure and medications, blood pressure, biochemical parameters (blood sugar and lipid profile) characteristics were examined and recorded for all individuals in two groups. The subject's blood pressure was measured at rest and after 5 minutes of rest, it was measured twice and the mean SBP and DBP were calculated.
Evaluation of ACE polymorphism: Evaluation of polymorphisms (I / D; rs4646994) of ACE gene was determined by PCR method and for this purpose, a venous blood sample was taken from all subjects. Part of the blood sample was transferred to a tube containing EDTA to extract the genomic DNA. DNA extraction from blood samples in the EDTA tube was performed by the standard salting out method.
PCR: Reproduction of genomic DNA fragments is performed in the intron16 area of the ACE gene. A pair of flanking primers was used for proliferation:
5'-CTG GAG ACC ACT CCC ATC CTT TCT-3'
5'-GAT GTG GCC ATC ACA TTC GTC AGAT-3'
Agarose gel electrophoresis: The proliferated parts were placed on the agarose gel 2% electrophoresis and the genotypes were determined under the UV light. Finally, the prevalence of polymorphisms (II, ID, and DD) in the ACE gene was compared in patients with hypertension and normal individuals.
Statistical analysis of data: The SPSS software version 22 was used for statistical analysis. The mean and standard deviation were used to describe the data in quantitative variables and the frequency and percentage were applied to discuss the qualitative variables. To analyze the data as a single variable, independent t-test (or Mann-Whitney nonparametric test), Chi-square test (or Fisher's precision), and Pearson correlation coefficient (or Spearman) were used. The level of significance in the above tests is P = 0.05 (95% confidence level).
Ethical considerations:

1) Obtaining a license from the Research Council and the Ethics Committee of Ahwaz Jundishapur University of Medical Sciences
2) Obtaining permission from the officials of Imam Khomeini Hospital in Ahvaz
3) Obtaining written and informed consent to participate in the study
4) No financial or physical damage was inflicted on the patients
5) The information in the patients' file will be completely confidential

RESULTS

In this study, which was performed on 40 patients with hypertension and 32 healthy individuals, the mean ages of the case and control groups were 50±8.54 and 45.5±7.54 respectively. The BMI in the case group was higher than the control group and there was a statistically significant relationship between BMI and the two groups. The results showed that the mean of SBP and DBP in the case group was much higher than the control group and a significant relationship was found between SBP and DBP in the two groups. In this study, 19 males and 21 females participated in the case group and 17 males and 15 females participated in the control group.

Using the independent t-test, a significant difference was observed between the mean age, BMI, SBP and DBP and hypertension of the case and control groups (P <0.05). However, there was no significant difference in

terms of gender in the two groups. There was a statistically significant difference between the allele frequency in the two studied groups (P <0.05).

The results of Table 1 show that the DD and DI polymorphisms of the ACE gene are higher in the control group, which was statistically significant.

The results of Table 2 showed that in the case group, the DD and DI polymorphisms were higher in women and men, and polymorphism II was the same in both genders. However, there was no statistically significant difference between gender and ACE polymorphism. The DD and DI polymorphisms were reported the same in women and men and there was no statistically significant difference.

The results of Table 3 showed that the DD, DI and II polymorphisms were less in the age group <40 than that of the >40 age group; however, no statistically significant difference was reported.The results of Table 4 show that the use of ACEI medicine was higher in DD polymorphism. Moreover, the use of nonACEI medicine was higher in polymorphism DI but it was not statistically significant.

The results of Table 5 showed that there was no statistically significant association between medication effect and ACE polymorphism, systolic and diastolic blood pressure.

Table 1- Determining and comparing the association of different types of ACE gene polymorphisms in the two studied groups

Group	ACE		
	DD	DI	II
Case	19(47.5%)	15(37.5%)	6(15%)
Control	3(9.4%)	16(50%)	13(40.6%)

P value 0.001

Table 2- Determining the distribution of different types of ACE gene polymorphisms in in terms of gender in the two groups

Group/ Gender	ACE			P
	DD	DI`	II	
Case				
Female	13(61.9%)	5(23.8%)	3(14.3%)	0.12
Male	6(31.6%)	10(52.6%)	3(15.8%)	
Control				
Female	0	9(60%)	6(40%)	0.2
Male	3(17.6%)	7(41.2%)	7(41.2%)	

Table 3- Determining the relationship between the types of ACE gene polymorphisms at different ages in the two studied groups

Group/ Gender	ACE			P
	DD	DI`	II	
Case				
<40	4(57.1%)	2(28.2%)	1(14.3%)	0.84
>40	15(45.5%)	13(39.4%)	5(15.1%)	
Control				
<40	0	6(54.5%)	5(45.5%)	0.42
>40	3(14.3%)	10(47.6%)	8(38.1%)	

Table 4- Determining the effect of medication, especially ACE inhibitors, on blood pressure control according to the type of polymorphism in the case group

Group/ Medication	ACE			P
	DD	DI`	II	
Case				
No medication	2(50%)	2(50%)	0	0.34
ACEI	9(60%)	4(26.7%)	2(13.3%)	
Non ACEI	6(28.6%)	10(47.6%)	5(23.8%)	

Table 5-Binomial regression related to the effect of medication on the factors affecting hypertension

Variable	β	S.E.	Wald	Df	p
ACE	0.7832	0.508	2.075	1	0.15
SBP	0.000	0.034	0.000	1	1
DBP	0.041	0.064	0.407	1	0.52
Constant	3.885	3.797	1.047	1	0.306

DISCUSSION

In the present study, 40 patients with hypertension were compared with 32 healthy individuals. The studied factors in this study were age, gender, BMI, systolic and diastolic blood pressure, ACE gene polymorphism and medications. Mean ages of the case and control groups were 50±8.54 and 45.5±7.54 respectively.

The results showed that the mean BMI, SBP and DBP in the case group was much higher than the control group and a significant relationship was reported between BMI, SBP and DBP in the two groups. The case group was divided into two groups of 37.5% controlled blood pressure and 62.5% uncontrolled blood pressure groups and 5 factors were addressed.

In this study 19 males and 21 females participated in the case group and 17 males and 15 females enrolled in the control group.

Using an independent t-test, there was a significant difference between mean age, BMI, SBP and DBP, and hypertension in the case and control groups (P <0.05). However, there was no significant difference in terms of gender between the two groups. There was a statistically significant difference between the allele frequency in the two studied groups (P <0.05).

The results indicate that the DD and DI genotypes of ACE polymorphism in the case group were higher than the control group and a statistically significant difference was obtained.The DD genotype was three times, DI genotype was twice higher than the genotype II in the patients with uncontrolled blood pressure among the groups of ACE polymorphism, and the DD and DI genotype were more than genotype II in the group of patients with controlled blood pressure. No statistically significant relationship was found between the types of ACE polymorphism and controlled and uncontrolled blood pressure in the case group.

The results of the present study showed that in the case group, the DD and DI polymorphism were higher in women and men respectively and polymorphism II was the same in both genders. The DD and DI polymorphism were higher in women and men respectively and polymorphism II was the same in both genders but there was no significant difference between the polymorphisms of the ACE gene in terms of gender in the two groups.

In the present study, the ACE polymorphism was lower in the age group <40 than that of the age group >40, however, no statistically significant difference was reported.

The results showed that ACEI medicine was more common in DD genotypes. Also, in the DI genotype, the use of nonACEI medicine was higher, which was not statistically significant. The results also showed that there was no statistically significant relationship between medicine effect and ACE polymorphism, systolic and diastolic blood pressure.

In the present study, the results of higher prevalence of individuals with DD genotype in the hypertensive group (47.5%) confirm the result of the study of Rana et al. (2018) in Indiathat shows a possible relationship between DD genotype and hypertension. Analysis of the odds ratio (OR) also showed a significant 2.225 (1.37-1.13) times increase in the risk of blood pressure in samples with DD genotyp (3). Therefore, this study showed an increased risk of hypertension due to the DD ACE genotype in the studied population.

In the present study, the results showed that the frequency of DD genotype in the case group (47.5%) was higher than the control group (9.4) and the frequency of gene typing ID in the control group was higher than the case group. The DD genotype was significantly associated with systolic blood pressure (SBP) ($P < 0.05$) in the case group. Generally, the results showed that there was a significant relationship between DD and higher SBP genotypes in patients with hypertension, which was consistent with Hussain et al. (2018) in Pakistan, Mannan et al. (2017) in India and Taleh Krishnan et al. (2016) in India[6,1,24]

The results of multivariate regression analysis of He et al. (2013) in China showed that the incremental model (ID, DD vs. II) of the ACE genotype had a significant relationship with hypertension (OR = 1.43; CI: 1.04-1.97) and this relationship was established for ACE ID genotype with OR = 1.72 (95% CI: 1.01-2.92) and DD genotype with OR = 1.94 (95% CI: 1.01-3.73). In addition, the results showed that there was a significant relationship between ACE plasma activity (OR = 1.13; CI: 1.08-1.18) and hypertension. However, no significant difference in plasma expression of ACE mRNA was observed between the case and control groups. As a result, the ACE I/D polymorphism and ACE activity had a significant effect on hypertension[25].

In the present study, 62.5% of the patients with high blood pressure had diastolic and systolic hypertension and in this group, the frequency of women was higher than that of the men (71.4 vs. 52.6%). The predominant DD genotype was 47.5% and the genotype II had the lowest prevalence of 31.6%. The frequency of D/D genotypes in hypertensive patients was significantly higher than the control group. Therefore, there was a significant relationship between Del / Del genotype and isolated and systolic hypertension. This result shows that D / D genotype of ACE gene plays a significant role in hypertension, which was inconsistent with the study of Borach et al. in India in terms of dominant and recessive. However, it was consistent with Choudhury et al. (2012) in India[27] in terms of the relationship between DD genotype and SBP[26].

In this study no significant relationship was reported between DI genotype and systolic blood pressure, which was consistent with Sudhir et al. (2012) in India[28].

CONCLUSIONS

The present study showed that primary hypertension is associated with the ACE polymorphisms.

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