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Association of Angiotensin-Converting Enzyme Inhibitor Gen PolyMorphism with Electrocardiography and Echocardiography Findings in Hemodialysis Patients

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Abstract

Background: Gene polymorphism of angiotensin-converting enzyme (ACE) may be associated with adverse prognosis and increased cardiovascular complications in hemodialysis patients.

Objectives: This study aimed to compare the frequency of ACE gene polymorphism in both hemodialysis patients and normal individuals considering echocardiographic findings.

Methods: This cross-sectional study included 110 hemodialysis patients (case) and 113 healthy subjects (control). Gene polymorphism of ACE was evaluated in both groups. ECG and echocardiography tests were performed for all patients. Correlations between gene polymorphisms and other variables were analyzed in this study. Polymerase chain reaction (PCR) was used to identify the short deletion allele (D with 190bp), large insertion allele (I with 490bp), and ID genotype which has both alleles.

Results: Case and control groups included 46 and 54 female and 64 and 59 male patients, respectively. There were no significant differences between the prevalence of DD, II, and DI alleles of the ACE gene with DI as the most common allele in both groups. No significant differences were found between systolic and diastolic blood pressure and heart rate in DD, DI, and II alleles of the case group. Echocardiographic findings of the patients showed no significant differences between DD, DI, and II genotypes of the case group and intraventricular septal end-diastole (IVSd), MVE vel, MVA vel, MVE/A ratio, MV DT, and MV Dec slope. The mean±SD left ventricular end-diastolic diameter (LVEDD) in II, ID, and DD patients were 4.3±0.72, 4.52±0.66, and 4.89±0.93 respectively (P=0.046).

Conclusion: The findings of the present study showed that there were no differences in the prevalence of alleles of an ACE gene in hemodialysis patients and control groups. Moreover, no significant associations were observed between alleles of an ACE gene in the patients' group and echocardiographic findings except in left ventricular end-diastolic diameter.

Keywords: Angiotensin-converting enzyme, Electrocardiography, Echocardiography, Gene polymorphism, Hemodialysis

1. Background

Chronic kidney disease (CKD), as a serious health problem, is defined as a permanent functional or structural abnormality of the kidneys and is often presented as functional abnormalities, such as proteinuria with no decrease in glomerular filtration rate (GFR). However, in CKD patients, GFR reduces gradually and leads to end-stage renal disease (ESRD) after several months to years (1). Moreover, GFR reduction and proteinuria increase the risk of cardiovascular disease in CKD patients. In addition, diabetes mellitus (DM) and hypertension, two major and common causes of CKD, also increase the risk of cardiovascular diseases (2,3). Furthermore, microvascular complications of DM, such as glomerulopathy, and macrovascular complications of DM, such as generalized atherosclerosis and cerebrovascular disease, may also increase cardiovascular diseases and overall mortality in these patients (4,5). Some other cardiovascular risk factors, including hyperuricemia, hyper-homocysteinemia, prolonged anemia, left ventricular hypertrophy, chronic inflammation and oxidative stress, secondary hyperparathyroidism, and bone mineral disorder in CKD patients are also important (6–9). Previous studies also investigated the association of polymorphism of different gens, such as angiotensin-converting enzyme (ACE), in CKD and hemodialysis patients (10–13).

Hypovolemia and salt intake lead to the secretion of renin via juxtaglomerular cells in the kidneys (14). Afterward, angiotensinogen, as an inactive protein, will be converted to angiotensin 1 by renin and then to angiotensin 2, which is a potent vasoconstrictor, by ACE. In addition, angiotensin 2 stimulates the adrenal cortex for the secretion of aldosterone (15). Moreover, ACE can also be secreted by vascular endothelial cells, lungs, and glomerulus (15).

Genes of the renin-angiotensin system (RAS) are highly prone to polymorphic induction, such as

insertion/deletion (I/D) polymorphism. In this regard, deletion polymorphism of the ACE gene was reported to be associated with the elevated serum ACE level (16). In a study performed on 916 patients with D/D polymorphism of ACE gene, there was an association with isolated systolic hypertension (17). Moreover, the DD genotype of ACE may also be associated with lower median renal survival and can worsen the renal prognosis in patients with the autosomal dominant polycystic disease (18). In addition, ACE DD genotype may also be related to the severity of proteinuria and progression of diabetic nephropathy into ESRD among type 2 diabetic patients (11). An ACE gene polymorphism may also be associated with rapid and more severe progression of renal failure in non-diabetic ESRD (19,20). Due to the controversy on the association between ACE gene polymorphism and cardiac manifestations of dialysis patients.

2. Objectives

This study aimed to compare the frequency of ACE gene polymorphism in hemodialysis patients with normal papulation and evaluate the association of D and I polymorphism of ACE gene with echocardiographic findings in the case group.

3. Methods

3.1. Sample collection

This cross-sectional study included 110 hemodialysis patients and 113 healthy people as two groups of case and control, respectively. Hemodialysis patients were chosen from the hemodialysis department of Hajar Hospital in Shshrekord, Iran, and the control group was selected from healthy volunteer subjects. Inclusion criteria were age over 18 years in both study groups and hemodialysis duration greater than 3 months in the case group. The exclusion criteria included the unwillingness to cooperate in the study and the history of renal and cardiac disease in the control group.

3.2. Measurements

Gene polymorphism of the ACE gene was evaluated in the case (hemodialysis patients) and control (healthy subjects) groups. The patients in the case group underwent both electrocardiography (ECG) and echocardiography. Polymerase chain reaction (PCR) was used to identify the short deletion allele (D with 190bp), large insertion allele (I with 490bp), and ID genotype which has both alleles.

Each PCR was performed in a 10 µL reaction containing the following reagents: 25 ng genomic DNA, 1 µL buffer (10 mMTris-HCl {pH 8.3}, and 1.5 mM MgCl2), 1% DMSO (SigmaAldrich, St. Louis, MO), 200 µMdNTPs, 0.5 µM insertion specific primer: 5′CTG GAG ACT CCC ATC CTT TCT 3′and 5′ GAC GTG GCC ATC ACA TTC GTC AGA 3′ and 1U Taq DNA polymerase (sinagen, Iran). In addition, the condition of PCR cycles included: initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94°C for 40 s, annealing at 62°C for 90 s, extension at 72°C for 50 s, and a final extension at 72°C for 3 min. The amplified genes were separated by 12.5% polyacrylamide gel electrophoresis and visualized by silver staining (21). Moreover, the sequences of forward and reverse primer were: 5′CTG GAG ACT CCC ATC CTT TCT 3′and 5′ GAC GTG GCC ATC ACA TTC GTC AGA 3′, respectively.

Moreover, ECG and echocardiography were applied to patients in the case group by the same cardiologist using an echocardiography device (GE Vivid S6 ultrasound system, USA).

3.3. Statistical analysis

Data were analyzed in SPSS software (Version 20.0, SPSS Inc., Chicago, IL, USA) using the Chi-square test for categorical data, student's t-test for comparing continuous variables between two groups of polymorphisms, and analysis of variance for comparing continuous variables among three groups of polymorphisms. All continuous variables were presented as mean±standard deviation (SD), and a p-value less than 0.05 ($P<0.05$) was considered statistically significant. All patients' information was kept confidential and informed written consent was taken from all participants at the beginning of the study. This study, with ethical code 92-3-21, was funded by the Research and Technology Deputy of the Shahrekord University of Medical Sciences, Shahrekord, Iran.

4. Results

There were 46 and 54 female and 64 and 59 male patients in the patients and control groups, respectively. The mean age of patients in the case and control groups were 57.61±15.57 years (21-87 years old) and 57.45±15.94 (24-82 years old), respectively. There were no significant differences between the two study groups in terms of the prevalence of DD, II, and DI alleles of the ACE gene (Table 1). The most

Table 1. Prevalence of ACE gen polymorphism in case and control group

Group	Gender	II	ID	DD	Total	P-value
Control	Men	2	34	22	58	0.871
	Women	3	32	20	55	
Case	Men	0	31	34	65	0.039*
	Women	3	26	16	45	
Total		8	123	92	223	0.40

*Significant difference

Table 2. Mean age of the patients (year) in the case and control groups

Group	Polymorphism	Age (Mean±SD)	
Control (men)	II	36.5±2.12	0.024*
	ID	61.88±13.32	
	DD	57.95±16.57	
	All	59.52±15.06	
Control (women)	II	46±15.52	0.36
	ID	53.13±16.41	
	DD	58.6±18.12	
	All	54.73±17.04	
Case (men)	II	-	0.94
	ID	58.42±18.09	
	DD	58.76±16.71	
	All	58.6±17.24	
Case (women)	II	41.67±15.95	0.02*
	ID	54.31±14.25	
	DD	62.63±10.03	
	All	56.42±13.88	

*. Significant differences P=0.81

Table 3. Correlation of gene polymorphism with some continuous variables in both genders

Variables	Gender	Polymorphism			All	P-value	P-value
		II	ID	DD			
SBP (mmHg)	Men	-	169 ±155.59	144.71± 21.35	155.62 ± 117.72	0.371	0.395
	Women	103.33 ±5.77	169.19 ±171.43	140.63 ±23.51		0.63	
DBP (mmHg)	Men	-	88.35±14.14	88.68±10.75	86.07 ± 13.67	0.918	0.012*
	Women	63.33±5.77	83.62±14.01	84.38±15.04		0.062	
PR interval	Men	-	75.52±7.89	77.65±6.48	76.87 ±7.15	0.237	0.977
	Women	76±3.46	78.5±7.95	75.38±5.97		0.377	
QTC interval	Men	-	0.44±0.073	0.44±0.04	0.44±0.05	0.75	0.254
	Women	0.4±0.02	0.43±0.04	0.45±0.05		0.106	
IVSd (cm)	Men	-	1.16±0.19	1.18±0.26	1.13±0.21	0.62	0.07
	Women	0.87±0.98	1.08±0.17	1.07±0.13		0.078	
LVEDD (cm)	Men	-	4.68±0.66	4.98±0.82	4.68±0.81	0.116	0.046*
	Women	4.3±0.72	4.32±0.61	4.69±1.14		0.38	
MVE vel (m/sec)	Men	-	4.33±19.2	0.89±0.29	1.82±10.12	0.3	0.624
	Women	0.78±0.32	0.87±0.26	0.79±0.28		0.613	
MV A vel (m/sec)	Men	-	0.88±0.27	0.96±0.23	0.95±0.25	0.226	0.552
	Women	0.89±0.23	0.98±0.27	1.02±0.23		0.7	
MV E/A Ratio	Men	-	0.97±0.43	0.97±0.41	0.95±0.44	0.967	0.803
	Women	0.95±0.06	0.98±0.56	0.80±0.32		0.479	
MV DT (mil/ sec)	Men	-	281.23±151.81	273.76±101.53	267.91±130.73	0.815	0.864
	Women	307.33±75.65	246.12±164.49	257.69±88.77		0.479	
MV Dec Slope	Men	-	3.55±1.67	3.68±1.99	3.84±2.10	0.776	0.397
	Women	2.5±1.41	4.61±2.03	3.77±3.003		0.765	

*Significant difference

SBP=systolic blood pressure, DBP=diastolic blood pressure, HR=heart rate, IVSd=intraventricular septal end-diastole, LVEDD=left ventricular end-diastolic diameter, MV E vel=Mitral valve peak early filling velocity, MV A vel=mitral valve late diastolic filling velocity, MV DT= Mitral valve deceleration time

common allele was DI followed by DD and II in both groups. There was also no association between age and gender with gene polymorphism in the case group (Tables 2 and 3).

Patients in the case group underwent both ECG and echocardiography by the same cardiologist. There were no significant differences between systolic and diastolic blood pressure and heart rate in the group of patients with DD, DI, and II alleles. Based on the results of ECG, there were no differences in PR interval and corrected QT (QTc) in different genotypes. Echocardiographic findings of the patients showed that there were no significant differences between DD, DI, and II genotypes of the patients with intraventricular septal end-diastole (IVSd), MVE vel, MVA vel, MVE/A ratio, MV DT, and MV Dec slope (Table 4). However, the mean ±SD left ventricular end-diastolic diameter (LVEDD) was obtained at 4.3±0.72, 4.52±0.66, and 4.89±0.93 in II, ID, and DD patients, respectively (P=0.046).

5. Discussion

Based on the obtained results, there were no differences in the prevalence of alleles of an ACE gene in hemodialysis patients and control groups, and no significant association was observed between alleles of the ACE gene of the patients and echocardiographic findings except for left ventricular end-diastolic diameter.

Consistently, in another study, Losito A et al. found no difference in the prevalence of ACE gene alleles in 160 patients with ESRD who received hemodialysis, compared with 169 normal cases; however, DD alleles were more common in the patients with cerebrovascular disease, compared to other dialysis patients (22). In a study conducted on 453 hemodialysis patients, Van F et al. found that ID genotyping of ACE is more common than DD and II alleles, indicating that DD genotype may increase mortality in these patients (23). In a study on 103 ESRD diabetic patients with hemodialysis, Hyeong Cheon Park et al. reported that DD alleles of the ACE gene were more common in diabetic patients, compared to non-diabetic patients, and concluded that gene polymorphism of DD of ACE gene might have a role in the progression of diabetic nephropathy (11). In addition, Femke VAN Der S B et al. in their study on 453 hemodialysis patients demonstrated that ID or DD gene polymorphism of ACE gene was associated with all-cause mortality in the patients (24). Moreover, in the study conducted by Vleming LJ et al. on 79 type 1 diabetic patients, it was found that the DD genotype of ACE might be associated with a twofold increase in ESRD risk (25). In another study conducted by Emanuela Lovati et al. on 327 control subjects and 260 ESRD patients, faster progression of renal failure was also been observed in DD genotyping of the ACE gene (26). Therefore, DD genotyping of the ACE gene may be associated with the poor response to angiotensin-converting enzyme inhibitors. In the same line, in the study performed on 81 patients with non-diabetic renal disease, van Essen GG et al. found that neither atenolol nor Enalapril prevented progressive decreases in renal function (27).

Pérez-Oller L et al. in their study on 155 autosomal dominant polycystic kidney disease (ADPKD) stated that the DD genotype of ACE could be correlated with worsening renal prognosis and less median renal survival; therefore, the majority of patients with DD genotype developed ESRD before the age of 50 (18). In addition, Marjan A. van Dijk et al. in their study on 70 ADPKD patients reported no association between ACE gene polymorphism and progression of renal disease towards ESRD (28).

Some studies have also been conducted on the association between gene polymorphism and echocardiographic findings. In this regard, Felipe Neves de Albuquerque in the study on 111 patients with systolic function showed that the DD genotype was independently associated with worse echocardiographic outcome, while the DI genotype with the best echocardiographic profile increased left ventricular ejection fraction and decreased left ventricular diameters (29). In the study on 176 hypertensive patients with diastolic dysfunction, Bahramali showed that the D allele of the ACE gene was associated with the development of LVH (30). Wang AY et al. in their study on 460 peritoneal patients demonstrated that ACE gene polymorphism was associated with more severe left ventricular hypertrophy in these patients (31). Moreover, in the study on ESRD patients undergoing hemodialysis, Osono E demonstrated that the DD genotype of the ACE gene was a risk factor for the development of LV hypertrophy (32). In addition, in the study on 106 diabetic patients with ESRD undergoing hemodialysis Sakka Y et al. showed that DD gene polymorphism of ACE gene had increased mortality but had no effect on left ventricular hypertrophy (33).

As mentioned above, there are some studies regarding the association of ACE gene polymorphism and echocardiographic findings in hemodialysis patients. The results of these studies showed that there might be an association between some alleles of ACD gene polymorphism and echocardiography abnormality. Regarding the limitations of this study, one can refer to the small sample size and lack of patients follow-up. It is recommended that further studies should be conducted with a larger sample size and long-time follow-up on these patients.

6. Conclusion

The findings of the present study showed that there were no differences in the prevalence of alleles of the ACE gene in hemodialysis patients and control groups. Moreover, there was no significant association between alleles of the ACE gene in the group of patients and echocardiographic findings except for left ventricular end-diastolic diameter.

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Footnotes

Conflict of Interests: The authors have no conflict of interest regarding the publication of this study.
Author's contributions: Dr. Ali Momeni and Alireza Nematolahi contributed to the conceptualization and methodology. Maryam Mohammadi collected the Data. Masoud Amiri performed data analysis and interpretation. Morteza Hashemzadeh Chaleshtori participated in drafting the article, and Mahdi Ghatrehsamani revised the manuscript.
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