

Relation between angiotensin-converting enzyme I/D gene polymorphism and pulse pressure in patients with a first anterior acute myocardial infarction

İlk kez anterior akut miyokard infarktüsli hastalarda anjiyotensin dönüştürücü enzim I/D gen polimorfizmi ile nabız basıncı arasındaki ilişki

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ABSTRACT

Objective: Evidence shows that an elevated pulse pressure (PP) may lead to an increased risk of cardiovascular morbidity and mortality. The aim of the present study was to determine the effects of polymorphism of the angiotensin-converting enzyme (ACE) gene on the PP after a first anterior acute myocardial infarction (AMI).

Methods: Overall 116 patients with a first anterior AMI were included in this cross-sectional study. DNA was isolated from peripheral leukocytes. The ID status was determined by polymerase chain reaction by a laboratory staff member who was unaware of the clinical details. Based on the polymorphism of the ACE gene, they were classified into 3 groups: Deletion/Deletion (DD) genotype (Group 1, n=45), Insertion/Deletion (ID) genotype (Group 2, n=58), Insertion/Insertion (II) genotype (Group 3, n=13). Blood pressure measurements were performed in all patients within 10 minutes admitted to coronary care unit. The PP was calculated by subtraction of diastolic blood pressure (DBP) from systolic blood pressure (SBP). Echocardiographic examinations were performed using the parasternal longitudinal axis and apical 4-chamber windows in accordance with the recommendations of the American Echocardiography Committee. One-way analysis of variance (ANOVA) and Chi-square analyses were used to compare differences among subjects with different genotypes.

Results: There were no significant differences among clinical parameters of patients. Pulse pressure was significantly higher in patients who have ACE DD and ID genotypes than in patients who have ACE II genotype (47 ± 16 , 47 ± 14 and 39 ± 12 , $F=3.4$, $p<0.05$). But SBP, DBP and heart rate were not significantly different among ACE DD, ACE ID and ACE II genotypes.

Conclusion: Our results suggested that, ACE Gene I/D polymorphism D allele may affect PP in patients with a first anterior AMI. (*Anadolu Kardiyol Derg 2009; 9: 9-14*)

Key words: Angiotensin converting enzyme, gene, polymorphism, pulse pressure, myocardial infarction

ÖZET

Amaç: Kanıtlar nabız basıncındaki artışın kardiyovasküler morbidite ve mortalite riskinde artışa yol açtığını göstermektedir. Bu çalışmanın amacı, ilk kez anterior akut miyokard infarktüs geçiren hastalarda, nabız basıncı üzerinde anjiyotensin dönüştürücü enzim (ACE) gen polimorfizminin etkilerini belirlemektir.

Yöntemler: Bu enine kesitli çalışmaya ilk kez anterior akut miyokard infarktüsü geçiren 116 hasta alındı. DNA periferik lökositlerden izole edildi. ID durumu, klinik bulgulardan habersiz laboratuvar üyesi tarafından, polimeraz zincir reaksiyonuyla belirlendi. ACE gen polimorfizmine göre hastalar 3 gruba ayrıldı. Delesyon/Delesyon (DD) Genotip (Grup 1, n=45), İnsersiyon/Delesyon (I/D) Genotip (Grup 2, n=58), İnsersiyon/İnsersiyon (II) Genotip (Grup 3, n=13). Kan basıncı ölçümleri hastalar koroner yoğun bakım ünitesine yatırıldıktan sonraki ilk 10 dk içerisinde ölçüldü. Nabız basıncı, sistolik kan basıncından diyastolik kan basıncının çıkarılması ile elde edildi. Ekokardiyografik inceleme Amerikan Ekokardiyografi Komitesi önerilerine uygun olarak, parasternal uzun aks ve apikal dört boşluk pencereler kullanılarak yapıldı. Farklı genotiplere sahip örnekler arasındaki farklılığı karşılaştırmak için ANOVA ve Ki-kare testleri kullanıldı.

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Bulgular: Klinik özellikler bakımından hastalar arasında fark yoktu. Nabız basıncı ACE DD ve ID genotipli hastalarda ACE II genotipli hastalara göre anlamlı derecede yüksek bulundu (sırası ile 47 ± 16 , 47 ± 14 ve 39 ± 12 , $F=3.4$, $p<0.05$). Fakat sistolik kan basıncı, diyastolik kan basıncı ve kalp hızı bakımından gruplar arasında anlamlı fark saptanmadı.

Sonuç: Yapmış olduğumuz bu çalışmada ACE Gen I/D polimorfizmi D alleli ilk kez anterior akut miyokard infarktüsü geçiren hastalarda nabız basıncını etkileyebileceğini gösterdik. (*Anadolu Kardiyol Derg 2009; 9: 9-14*)

Anahtar kelimeler: Anjiyotenzin dönüştürücü enzim, gen, polimorfizm, nabız basıncı, miyokard infarktüsü

Introduction

Cardiovascular (CV) mortality is not only positively correlated to the level of systolic blood pressure (SBP), but also at any given value of SBP, CV mortality is higher when diastolic blood pressure (DBP) is lower, particularly for subjects over 60 years of age (1). In recent years, evidence has accumulated that increased pulse pressure (PP) predicts CV mortality and coronary artery disease, myocardial infarction (MI) and congestive heart failure, independent of DBP and SBP, and other risk markers (2, 3).

Pulse pressure (PP), defined as the difference between the arterial SBP and DBP, is determined both by cardiac and vascular factors. Pulse pressure has been found to be a heritable trait and there is evidence that genetic factors influence the interindividual variation in PP. Genetic factors play a greater role in determining PP (4, 5).

Previous research findings on PP suggest that: (1) PP is an important predictor of CV disease and mortality, (2) genes appear to influence PP and (3) gender may be an effect modifier in the relation between PP and CV disease and mortality (5). In 1991, Hubert et al. (6) reported that the human angiotensin-converting enzyme (ACE) gene is localized in the long arm of chromosome 17 and that it consists of 26 exons and 25 introns. There is an I/D polymorphism in the Alu-like arrangement at intron 16 and this polymorphism correlates with the blood ACE concentration. The ACE gene explains 30-50% of its variance (6, 7). The ACE DD genotype has been associated with an increased risk of high blood pressure and MI in some studies, though this association is not consistently present (8, 9).

The relation between ACE gene polymorphism and PP in patients with a first anterior acute myocardial infarction (AMI) has not been reported previously.

Therefore, the objective of this study was to investigate the relation between ACE gene polymorphism and PP in patients with a first anterior AMI.

Methods

Subjects

This cross-sectional study included 125 consecutive patients (100 men, 25 women) who were admitted to the coronary care unit with anterior AMI, defined as (1) creatine kinase (CK) ≥ 210 IU/L and CK-MB ≥ 20 IU/L or (2) electrocardiographic evidence of MI (ST elevation >1 mm), and (3) typical chest pain. Patient's mean age was 59 ± 12 years. All patients were in sinus rhythm. Exclusion criteria were valvular heart diseases, atrial fibrillation, old MI, previous antihypertensive treatment, inadequate Doppler recordings and chronic obstructive pulmonary disease. Nine patients were excluded because of aortic stenosis (n=1), atrial fib-

rillation (n=2), old MI (n=3), previous antihypertensive treatment (n=2) and inadequate Doppler recordings (n=1), leaving a total of 116 patients. Based on the results of ACE gene polymorphism analysis, the patients were classified into 3 groups: group 1 (n=45)-DD genotype; group 2 (n=58)-ID genotype; group 3 (n=13)-II genotype. Patient characteristics are summarized in Table 1. The study protocol was approved by the ethics committee of our institution and informed consent was obtained from all patients.

Blood Pressure Measurement

Blood pressure measurements were performed in all patients within 10 minutes admittance to coronary care unit. Blood pressure was measured with a mercury sphygmomanometer after 5 minutes of rest, as recommended by the 6th Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure (10). Patients were seated with their arm bared and supported at heart level. Two readings, separated by 2 minutes, were obtained and averaged. Additional readings were obtained if these readings differed by >5 mm Hg. Pulse pressure was calculated by subtraction of diastolic blood pressure from systolic blood pressure. Mean blood pressure was calculated by the addition of two thirds of the PP to diastolic blood pressure. Body mass index was calculated by dividing the weight in kilograms by the square of the height in meters.

Treatment

Patient's blood pressure was measured after admittance to coronary care unit and then all patients were treated with thrombolytic therapy (streptokinase 1.5 million units/30 min or tissue type plasminogen activator 100 mg according to the accelerated protocol), acetylsalicylic acid-100, β -blocker (metoprolol 50-100 mg po) and intravenous nitroglycerin. The ACE inhibitor (silazapril 2.5-5 mg) or angiotensin-receptor blocker (valsartan 80-160 mg) was added to the treatment in the first 24 h, if there was no contraindication. Patients who have antihypertensive treatment at time of MI were excluded from the study.

DNA Analysis

DNA was isolated from peripheral leukocytes by the method described previously (11). The ID status was determined by polymerase chain reaction by a laboratory staff member who was unaware of the clinical details. The DD genotype of the ACE gene was reconfirmed by a second PCR using a Taq extender (Fig. 1) (12).

Echocardiography

Echocardiography was performed by one examiner (O.O.) within 24 h of arrival at the coronary care unit with a VingMed CFM 800 (Vingmed Sound, Norway) ultrasonographic machine with a 2.5 and 3.25-MHz transducer. Analyses were done blinded for all clinical data. All examinations were performed using the

Table 1. Clinical characteristics of patients according to I/D ACE Genotype

Parameters	ACE DD (n=45)	ACE ID (n=58)	ACE II (n=13)	p*
Age , years	58±11	59±12	56±14	NS
Gender, F/M	5/40	13/45	2/11	NS
BMI, kg/m ²	22±3	23±3	24±3	NS
Hypertension, n(%)	14 (33)	10 (18)	3 (21)	NS
Diabetes Mellitus, n(%)	4 (10)	4 (8)	0 (0)	NS
Current Smoking, n(%)	25 (57)	32 (56)	10 (78)	NS
Hypercholesterolemia, n(%)	9 (20)	17 (29)	3 (21)	NS
CK peak, IU/L	3128±2110	3077±2073	3075±1183	NS
CK-MB peak, IU/L	465±373	482±381	514±351	NS
MI localisation, n(%)				NS
1) Anteroseptal	7 (15)	11 (18)	2 (14)	
2) Anterior	17 (37)	19 (32)	3 (21)	
3) Large Anterior	30 (66)	39 (67)	9 (64)	
4) Anterolateral	2 (4)	3 (5)	0 (0)	
Thrombolytic Therapy, n(%)				NS
1) None	11 (24)	13 (22)	2 (14)	
2) STK	24 (53)	25 (43)	7 (50)	
3) t-PA	25 (55)	30 (51)	5 (35)	

* : Continuous variables are represented as mean±SD, categorical variables are displayed as percentages/proportions. * - p values for one-way ANOVA analysis and Chi-square test
ACE - angiotensin-converting enzyme, BMI- body mass index, BP- blood pressure, CK-creatinine kinase, MI - myocardial infarction, NS - not significant, STK - streptokinase, t-PA - tis-
sue plasminogen activator

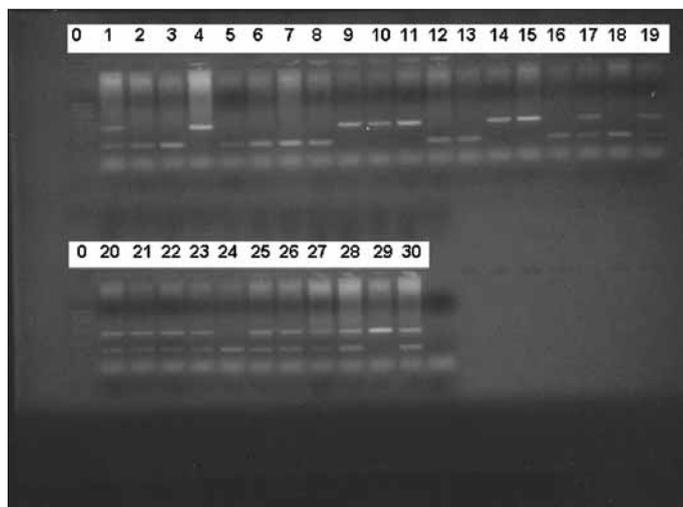


Figure 1. Gel electrophoresis of the ACE DI polymorphism. 0: a DNA size marker (100bp) ,1:ID, 2:DD, 3:DD, 4:II, 5:DD, 6:DD, 7:DD, 8:DD, 9:II, 10:II, 11:II, 12:DD, 13:DD, 14:II, 15:II, 16:DD, 17:ID, 18:DD, 19:ID, 20:ID, 21:ID, 22:ID, 23:ID, 24:DD, 25:ID, 26:ID, 27:ID, 28:ID, 29:II, 30:ID

parasternal longitudinal axis and apical 4-chamber windows in accordance with the recommendations of the American Echocardiography Committee (13). Using the parasternal long-axis view to assess the ventricular dimensions, the ejection fraction (EF) was calculated by the modified Simpson formula. Mitral inflow was recorded with the transducer in the apical 4-

chamber view. The pulsed wave Doppler beam was aligned as perpendicular as possible to the plane of the mitral annulus. The Doppler sample volume was placed between the tips of mitral leaflets during diastole. The left ventricular outflow velocity curve was recorded from the apical long-axis view with the Doppler sample volume positioned just below the aortic valve. Five consecutive beats obtained during quite respiration were measured and averaged for each Doppler variable and the peak velocities during early (E) and atrial contraction (A) filling, their ratio (E/A), deceleration time E (dtE) and isovolumic relaxation time (IVRT) for transmitral Doppler flows were assessed.

Statistical Analysis

Data were analyzed using the SPSS 10.0 for Windows (Chicago, IL, USA). Our data showed normal distribution. One-way analysis of variance (ANOVA) and Chi-square analyses were used to compare differences among subjects with different genotypes. Correlations between each parameter were examined by bivariate correlation test. Data are presented as mean±SD. A probability value <0.05 was considered significant.

Results

Clinical Parameters

Age, gender, heart rate, hypertension, body mass index, hypercholesterolemia, diabetes mellitus, smoking, the peak CK and CK-MB concentrations, diagnosis and treatment showed no significant differences among the 3 groups (Table 1).

ACE Gene I/D Polymorphism

Analysis of ACE gene polymorphism showed that 45 patients had the DD genotype, 58 had the ID genotype and 13 had the II genotype (Table 2). The observed prevalences of the ACE genotypes agree with the frequencies predicted by the Hardy-Weinberg equilibrium.

Blood Pressure Parameters

Pulse pressure was significantly higher in patients who have ACE DD/ID genotype than in patients who have ACE II genotype ($p<0.05$). But SBP, DBP and heart rate were not significantly different among ACE DD, ACE ID and ACE II genotypes (Table 3, $p>0.05$). There was a significant correlation between age and heart rate ($p<0.05$). There was no significant correlation between the pulse pressure and other clinical characteristics (Table 4).

Echocardiographic Findings

Echocardiography was performed in 116 patients at a median of 6 h (range 0-24 h) after arrival at the coronary care unit. There was no significant differences among the three groups according to echocardiographic parameters (Table 5, $p>0.05$).

Discussion

Our study confirms the presence of ACE gene polymorphism in patients with AMI and shows that, in the presence of the D allele, there is a steeper increase of PP with AMI than in patients who have ACE I allele. To our knowledge, few data have addressed the relationships between PP and gene polymorphism related to the renin-angiotensin system in rats (31). In hypertensive subjects, the present results suggest that the D variant of the ACE gene polymorphism contributes to modulation of the BP pulsatility in patients who have AMI.

Table 2. Angiotensin-converting enzyme genotypes and allele frequencies in patients with a first anterior AMI

ACE Genotype, n(%)	Genotype Frequency
DD	45 (38.8)
ID	58 (50)
II	13 (11.2)
ACE Alleles, n(%)	Allele Frequency
D Allele	148 (63.8)
I Allele	84 (36.2)

Data are represented as percentages/proportions.
Frequencies are computed using Chi-square test
ACE - angiotensin-converting enzyme

At any given value of SBP, CV mortality is higher when the DBP is lower (15). In fact, the predictive power of PP might arise from two different mechanisms. Increased SBP increases end-systolic stress and promotes cardiac hypertrophy (16, 17), whereas reduced DBP reduces coronary perfusion promoting myocardial ischemia and is associated with increased CV risk (18, 20).

In most previous reports on the genetics of hypertension, the hypothesis that genetic variability could lead to hypertension had been tested on the basis of comparison of mean DBP values in patients with different genotypes (21). The classification of hypertensive subjects was based on the measurement of DBP, a single point on the BP curve, whereas SBP and PP, the two mechanical factors which have the higher predictive value in term of CV risk, were often neglected in studies (22-23). Using this well-established procedure, negative results were observed in the present investigation, as previously observed in many other reports (24). Our principal goal was to test the hypothesis that there is a relation among the ACE Gene I/D polymorphism and SBP, DBP, PP in patients with MI.

It has been reported that ACE polymorphism with the DD genotype is a risk factor for the development of MI (7), hypertension (25), cardiomyopathy (26), cardiac hypertrophy (27), and restenosis following percutaneous angioplasty (28,29). In our previous study we have found that the ID polymorphism of the ACE gene may affect right and left ventricular performance indexes after a first anterior AMI (30). However, Tseng et al. (31) reported no significant association between the ACE genotype and peripheral vascular disease in Chinese type 2 diabetic patients.

This study examined relation among the ACE gene polymorphism and SBP, DBP, PP in patients with first anterior AMI. We have found that patients who have ACE D allele have higher PP than patients who have ACE I allele.

Table 4. Correlation of patients' characteristics and pulse pressure

	r	p
Age, years	0.235	0.004
Gender	0.090	NS
BMI, kg/m ²	0.026	NS
Heart Rate, beats/min	0.164	0.048
Diabetes Mellitus	0.152	NS
Smoking	0.034	NS
Hypercholesterolemia	-0.082	NS

* - Pearson correlation analysis
BMI - body mass Index, NS - not significant

Table 3. Blood pressure parameters of patients according to I/D ACE genotype

Variables	ACE DD (n=45)	ACE ID (n=58)	ACE II (n =13)	F*	p*
Systolic BP, mm Hg	129±29	127±22	120±17	0.650	NS
Diastolic BP, mm Hg	82±17	80±13	81±10	0.679	NS
PP, mm Hg	47±16	47±14	39±12	3.401	<0.05
Heart Rate, bpm	88±21	85±17	78±14	1.429	NS

Continuous variables are represented as mean±SD, categorical variables are displayed as percentages/proportions. * - p values for one-way ANOVA analysis and Chi-square test
ACE - angiotensin-converting enzyme, BP- blood pressure, NS - not significant, PP - pulse pressure

Table 5. Echocardiographic parameters of patients according to I/D ACE genotype

Variables	ACE DD (n=45)	ACE ID (n=58)	ACE II (n=13)	p*
Mitral E/A	1.0±0.39	0.93±0.35	1.04±0.22	NS
Mitral DT, ms	151±43	155±48	163±25	NS
Mitral IVRT, ms	89±21	98±29	96±20	NS
IVSd, cm	1.01±0.18	1.04±0.18	0.97±0.15	NS
LVDD, cm	4.94±0.41	4.84±0.59	4.92±0.54	NS
LVPWd, cm	0.93±0.19	0.94±0.17	0.91±0.16	NS
IVSS, cm	1.12±0.18	1.13±0.16	1.09±0.15	NS
LVDS, cm	3.96±0.52	3.85±0.59	3.6±0.65	NS
LVPWS, cm	1.07±0.14	1.09±0.16	1.05±0.13	NS
LVEF, %	41±6	41±7	43±4	NS

Data are represented as mean ±SD.
* - One-way ANOVA analysis
A- peak A wave velocity, ACE - angiotensin-converting enzyme, DT- mitral deceleration time, E- peak E wave velocity, IVRT- isovolumic relaxation time, IVSD- interventricular septum diastolic thickness, IVSS- interventricular septum systolic thickness, LVDD- left ventricular diastolic diameter, LVEF- left ventricular ejection fraction, LVPWD- left ventricular posterior wall thickness, LVSD- left ventricular systolic diameter, NS- not significant

Although the ACE gene I/D allelic variant in humans has been implicated in arteriosclerotic CV disease, cardiac hypertrophy, restenosis and progression of diabetic renal disease, its role in the mechanism of PP in MI has remained difficult to evaluate (1). O'Donnell et al. (32) found evidence for association and genetic linkage of the ACE gene with hypertension and blood pressure in men, but not in women, when they analyzed over 3000 participants from the Framingham Heart Study.

The finding that the DD genotype might influence arterial pulsatility is difficult to interpret. From association and linkage studies, there is strong evidence that the ACE D allele accounts for almost half of the variance in ACE plasma levels (33). Our results provide an interesting contribution to this problem because the D allele might contribute to the increase of pulsatility in patients with a first AMI. Pharmacological studies indicate that the ACE Gene I/D polymorphism influences not only angiotensin II generation but also the cross-talk of this hormone with bradykinin and even nitric oxide (34). It seems likely that the combination of all these vasoactive compounds changes with age and contributes in turn to the age-related changes in arterial stiffness and thus, in PP in subjects with DD genotype. Furthermore, the present findings agree with reports suggesting the influence of the ACE gene polymorphism on the mechanisms of blood pressure (35).

Study Limitations

Our study is the first to investigate the relationship between ACE gene I/D polymorphism and pulse pressure in patients with a first anterior AMI. For this reason, there are no data in the literature to compare our results. In this study, invasive hemodynamic measurements were not available. Angiography with assessment of artery patency was not performed routinely. Reperfusion rates were not measured.

Conclusions

Finally, the present investigation has shown that the ACE Gene I/D polymorphism D allele may modulate the relationship

between AMI and PP. This finding might play a role in the mechanism of CV risk. Clearly these results require further investigation involving long-term follow-up.

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