ACE insertion/deletion polymorphism and restenosis events after coronary stent placement in East Azerbaijan Province of Iran

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Abstract

Renin-angiotensin system is thought to play a role in coronary thrombosis and restenosis. Plasma angiotensin I-converting enzyme (ACE) activity is associated with an insertion/deletion polymorphism in the gene coding for ACE. The association among restenosis within coronary stents, the D/I polymorphism is analyzed in the present study. We are selected 50 patients who had undergone successful percutaneous transluminal coronary angioplasty, and angiography during 1 year after it for some reason. The I/D alleles were identified on the basis of polymerase chain reaction (PCR) amplification of the respective fragments from intron 16 of ACE gene. The differences between groups were tested by Chi-square test or Fisher exact test. Result from chi-square test and Fisher exact test shows the lack of differences between two groups with respect to sex, age, diabetes, hypertension, cholesterol, hyperlipidemia, stent type, length of stented segment, stent diameter and target vessel. In the second set of analysis, the relationship between genotypes and the outcome variable was tested. These analyses indicated that there were no statistically significant differences the three groups ($\chi 2$ (2) = 1.40, p=0 .47). The ACE D/D genotype or D allele does not influence the clinical and angiographic outcome of patients undergoing coronary stent placement. These data suggest that routine determination of the ACE genotype may not help identify patients who are at a higher risk of thrombotic and restenosis events after coronary stent placement.

Keywords: Thrombosis, restenosis, angiographic, stent, ACE, gene

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Introduction

Coronary artery disease (CAD) and myocardial infarction (MI), is a complex disorder resulting from the interaction between genetic and environmental factors [1,2]. Percutaneous transluminal coronary angioplasty (PTCA) was introduced in 1979 [2]. Restenosis after primary successful percutaneous coronary interventions (PCIs) with balloon angioplasty is one of the principal wound healing response to vascular wall trauma, occurring in up to 50% of patients undergoing the procedure without stenting and in about 20% of patients receiving stents. The molecular mechanisms of the arterial remodeling are less well understood. Even with extensive efforts using the genome-wide linkage studies, the responsible genetic determinants remain largely uncategorized [2-4]. Genetic epidemiology might provide insights into the pathophysiology of coronary restenosis and easily identifiable markers for predicting an increased restenosis risk [2,4,5]. Despite a lack of good evidence that susceptibility to restenosis is genetically determined, several studies have investigated polymorphisms that might be associated with restenosis [2,3,5-9]. Among the most studied genes for its association in pathogenesis of CAD and related outcomes is angiotensin converting enzyme (ACE) gene, located on chromosome 17q23. The genetic polymorphism in intron 16, characterized by an insertion (I) or a deletion (D) of a 287 noncoding base pair Alu repeat sequence (rs4646994) has been correlated with the levels of circulating and intracellular ACE [3,4,10,11].

The DD genotype of the angiotensin I– converting enzyme (ACE) gene is associated with higher angiotensin II levels, suggesting that carriers of the DD genotype might benefit from treatment with ACE inhibitors after stent placement in coronary arteries (11-14).In the light of the increasing number of patients being treated with multiple percutaneous coronary interventions, the increasing cost of interventional cardiology due to adjuvant medication and devices, and the difficulty in treating in-stent restenosis, it would be useful to define subsets of patients at increased risk for restenosis (2;11;12;14-16). The aim of this study is to explain the available ACE I/D in North West of IRAN and response-to-treatment studies for CAD and to quantify the estimated risk associated with this polymorphism.

Materials and Methods

Selection of Samples and Study Design

Between December 2011 and December 2013, 50 patients with successful percutaneous coronary interventions (PCIs) and complete clinical and angiographic follow-up were enrolled, at Shahid Madani Hospital, Tabriz, IRAN. All 50 eligible patients were requested to undergo a follow-up angiography 12 months after the procedure by attending physicians who were unaware of the patient's ACE D/I genotype. Patients who were undergoing angioplasty, blood samples and DNA extraction was performed from them.

Polymerase Chain Reaction for Detection of the ACE D/I Genotype

A detailed description of the protocol used to determine ACE D/I genotype has previously been published. In brief, the D and I alleles were identified on the basis of polymerase chain reaction (PCR) amplification of the respective fragments from intron 16 of ACE and by subsequent electrophoretic size fractionation and visualization after staining with gel red. Because the D allele tends to be preferentially amplified in heterozygotes, all samples determined to be DD were subjected to a second independent PCR amplification with a primer pair that recognizes an insertion-specific sequence to ensure accurate genotyping. To confirm genotype assignment, the PCR procedure was performed on all samples on 2 separate occasions. PCR condition, cycling and composition are shown in Tables 1 and 2 respectively.

Statistical analysis

Variables were presented as frequencies and percentages (%).The primary data analysis was performed to declare the differences between patients with restenosis and without restenosis upon demographic and angiographic characteristics. The differences between groups were tested by Chi-square test or Fisher exact test. ACE genotypes were also assessed by some of demographic and angiographic characteristics. Small samples logistic regression analysis was performed to identify the ACE genotypes association with restenosis. Results from the logistic regression analyses were represented as odds ratios (ORs) and its 95% confidence intervals (CIs). Descriptive and analytic analyses of this article were conducted using SPSS software version 16.0. For all analysis the value of $p < 0.05$ was considered as statistically significantly and all tests of significance were two tailed.

Results

Table 3 presents the baseline characteristics of the 50 patients where a high proportion of participants (62.0%) developed restenosis. Result from chi-square test and Fisher exact test shows the lack of differences between two groups with respect to sex, age, diabet mellitus (DM), hypertension (HTN), cholesterol, hyperlipidemia (HPL), Familial hypercholesterolemia (FH), stent type, PCI, length of stented segment, stent diameter and target vessel.

Although the independent variables show no statistical differences, Patients with restenosis were more likely to be male (OR = 1.67), had BMS stent type (OR=1.70), had stented segment >=20 mm (OR=1.86) and had stents with a diameter of >=3 mm (OR =1.93).

In present study 8(25.8%) of 31 patients who develop restenosis, were hypertensive and there was a statistically significant relationship between PCI and HTN, so that most of hypertensive patients (50.0%) developed restenosis in 6-12 months, ($p<0.05$). The left anterior descending (LAD) was the target vessel for stent placement in 36 (72%) right coronary artery (RCA) in 8 (16%) and left circumflex coronary artery (LCX) in 6 (12%) cases.

Other demographic and Angiographic characteristics are presented in tables 3. In the second set of analysis, the relationship between genotypes and the outcome variable was tested. These analyses indicated that there were no statistically significant differences the three groups ($\chi 2$ (2) = 1.40, p=0 .47). Heterozygote (I/D) participants were 1.31 times more likely to exhibit restenosis than normal cases (95.0% CI for ORss € (0.39, 3.97)). Homozygote (D/D) participants were 1.75 times more likely to exhibit restenosis than normal cases (95.0% CI for ORss € (0.340, 5.99)). Homozygote (D/D) participants were 1.08 times more likely to exhibit restenosis than Heterozygote (I/D) cases (95.0% CI for ORss € (0.29, 4.39)) which were not statistically significant.

Table 1. List of primers that used in this study

Polymorphism	Primer Name	Primer Sequence (5' → 3')	PCR Product (*bp*)	Annealing Tm	Ref
ACE I/D	*ACE-Ins*	GAC CTG CTG CCT ATA CAG T	234	63	12
	ACE-Del	GAT TAC AGG CGT GAT ACA GT			
	ACE-Com	GGG TAA AAC TGG AGG ATG GCT C			
Beta Actin (IC)	*BETA-F*	CTAGAAGCATTGCGGTGGACGA TGG	650	61	13
	BETA-R	TGACGGGGTCACCCACACTGTGC CC			

Table 2. PCR cycling that used in this study

Cycle	Stage	Temperature (Centigrade)	Time (Min & Second)
ACE(intron 16 insertion/deletion)			
1	First Denaturation	**95**	*5 min*
10	First Cyclic Denaturation	**95**	*30 Sec*
	First Annealing & Extension	**63**	*50 Sec*
25	Final Cyclic Denaturation	**95**	*30 Sec*
	Final Annealing	**58**	*30 Sec*
	First Extension	**72**	*30 Sec*

Table 3. Demographic and Angiographic characteristics of 50 participants.

variables		levels	N (%)	Restenosis		Test Result
				No N (%) 19(38.0)	Yes N (%) 31(62.0)	
Gender		Males Females	29(58.0) 21(42.0)	9(47.4) 10(52.6)	20(64.5) 11(35.5)	$\chi^2(1) = 1.422$ p-value=0.233*
Age		<= 60 > 60	30(60.0) 20(40.0)	11(57.9) 8(41.2)	19(61.3) 12(38.7)	$\chi^2(1) = 0.057$ p-value=0.812*
Diabetes Mellitus		No Yes	33(66.0) 17(34.0)	12(63.2) 7(36.8)	21(67.7) 10(32.3)	$\chi^2(1) = 0.110$ p-value=0.740*
Smoking		No Yes	39(78.0) 11(22.0)	16(84.2) 3(15.8)	23(74.2) 8(25.8)	$\chi^2(1) = 0.689$ p-value=0.498**
HTN		No Yes	38(76.0) 12(24.0)	15(78.9) 4(21.1)	23(74.2) 8(25.8)	$\chi^2(1) = 0.146$ p-value=0.748**
Cholesterol		No Yes	49(98.0) 1(2.0)	19(100.0) 0(0.0)	30(96.8) 1(3.2)	$\chi^2(1) = 0.625$ p-value>0.999**
HPL		No Yes	31(62.0) 19(38.0)	11(57.9) 8(42.1)	20(64.5) 11(35.5)	$\chi^2(1) = 0.219$ p-value=0.640*
FH		No Yes	48(96.0) 2(4.0)	18(94.7) 1(5.3)	30(96.8) 1(3.2)	$\chi^2(1) = 0.127$ p-value>0.999**
Stent Type	Stent1	BMS DES	34(68.0) 16(32.0)	11(57.9) 8(42.1)	23(74.2) 8(25.8)	$\chi^2(1) = 1.438$ p-value=0.230*
	Stent2	BMS DES	7(43.8) 9(56.2)	1(25.0) 3(75.0)	6(50.0) 6(50.00	$\chi^2(1) = 0.762$ p-value=0.585**
	Stent3	BMS DES	1(50.0) 1(50.0)	0(0.0) 0(0.0)	1(50.0) 1(50.00	
PCI		<6 Months 6-12 Months >12 Months	13(26.0) 12(24.0) 25(50.0)	8(42.1) 4(21.1) 7(36.8)	5(16.1) 8(25.8) 18(58.1)	$\chi^2(2) = 4.229$ p-value=0.121**
Length of stented segment	Stent1	>20 <20	39(78.0) 11(22.0)	13(68.4) 6(31.6)	26(83.9) 5(16.1)	$\chi^2(1) = 1.639$ p-value=0.293**
	Stent2	>20 <20	5(33.3) 10(66.7)	2(50.0) 2(50.0)	3(273) 8(72.7)	$\chi^2(1) = 0.682$ p-value=0.560**
	Stent3	>20 <20	0(0.0) 2(100.0)	0(0.0) 0(0.0)	0(0.0) 2(100.0)	
Stent Diameter	Stent1	>3 <3	25(50.0) 25(50.0)	7(36.8) 12(63.2)	18(58.1) 13(41.9)	$\chi^2(1) = 2.122$ p-value=0.145*
	Stent2	>3 <3	5(33.3) 10(66.7)	2(50.0) 2(50.0)	3(27.3) 8(72.7)	$\chi^2(1) = 0.682$ p-value=0.560**
	Stent3	>3 <3	0(0.0) 2(100.0)	0(0.0) 0(0.0)	0(0.0) 2(100.0)	
Target Vessel	Stent1	LAD RCA LCX	36(72.0) 8(16.0) 6(12.0)	14(73.7) 2(10.5) 3(15.8)	22(71.0) 6(19.4) 3(9.7)	$\chi^2(2) = 0.953$ p-value=0.658**
	Stent2	LAD RCA LCX	8(50.0) 5(31.2) 3(6.0)	1(25.0) 2(50.0) 1(25.0)	7(58.3) 3(25.0) 2(16.7)	$\chi^2(4) = 1.378$ p-value=0.772**
	Stent3	LAD	2(100.0)	0(0.0)	2(100.0)	

*Pearson Chi-Square Test, ** Exact Test

Table 4. Gene polymorphisms and risk of restenosis

Protein	Polymorphism	Allele frequency	Reference	Procedure	Association	
	Likelihood	Magnitude				
Angiotensin converting enzyme	Deletion	0.53(0.51-0.54)	7	PTCA	Possible	3-4
Platelet glycoprotein Ia	C807T	0.40(0.39-0.42)	13	PTCA	Unlikely	1
Apolipoprotein E	ε4	0.16(0.12-0.21)	13	Stenting	Probably not	1-10

Discussion

Restenosis after PTCA procedures is assumed to be a multifactorial and multi-genetic process. The identification of gene polymorphisms associated with restenosis can provide possible targets for gene therapy [8,11-17]. The issue of the magnitude of the impact of common genetic polymorphisms on a complex disease such as CAD or post-angioplasty restenosis is contentious [2,12,15]. We should remember here that these diseases are probably a result of the individual exceeding some notional threshold for a number of deleterious factors. Most risk factors for such common diseases will actually be so-called 'intermediate traits' that show quantitative variation, so that people with enough such traits in the top tail of the normal distribution are likely to be at increased risk of the disease. The risk of restenosis following PTCA-balloon was consistent for the allele contrast, the recessive, the dominant and additive models, though the results showed significant heterogeneity (Table 4).

Heterogeneity may result from differences in sample selection (e.g., in age-at-onset, gender, or diagnostic criteria), in genotyping methodology (two different genotyping procedures were used), or may be due to real differences in populations (e.g., 'racial' descent) or due to interactions with other unknown risk factors (2,12,15,16,18,19]. In an Australian Caucasian population undergoing elective PTCA of one or more previously untreated coronary arteries, the DD genotype was observed with greater frequency than in a control population without coronary artery disease. These patients were included in a clinical trial evaluating the effect of low dose aspirin on quantitative angiographic determined restenosis [2,20]. Many studies have tried to characterize the effects of ACE I/D polymorphism on the response to treatment

in CAD, in the context of both interventional and conservative therapeutic options for clinical and surrogate endpoints [2,21]. However, the reported results so far are discrepant and inconsistent. In view of available evidence, genetic testing of ACE I/D polymorphism prior to clinical decision making is not currently justified. The relation between ACE genetic variation and response to treatment in CAD remains an unresolved issue. The results of long-term and properly designed prospective studies hold the promise for pharmacogenetically tailored therapy in CAD.

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