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Original Article

Role of the -786T>C Variant of the Endothelial Nitric Oxide Synthase Gene in Stent Restenosis Following Coronary Stent Deployment

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Abstract

Background: The role of polymorphisms on the sequences of the endothelial nitric oxide synthase (eNOS) gene has been proposed to predispose coronary artery disease patients to stent restenosis. We conducted the present study to examine the involvement of the role of the -786T>C *variant* of the eNOS gene in stent restenosis following coronary stent deployment.

Methods: This cross-sectional study was conducted on 100 consecutive patients who underwent coronary stenting. The study population was assigned into a case group, who had restenosis and were candidated for revascularization (n, 50), and a matched control group, who underwent coronary stenting but without evidence of restenosis within 6 months of stenting (n, 50). The -786T>C polymorphism was identified, following polymerase chain reaction (PCR), by restriction enzyme digestion.

Results: The overall prevalence of restenosis was 34.4%. In total, the frequency of the wild genotype (CC) of the -786T>C variant was 41.9%, the frequency of heterozygous genotype (TC) was 41.9%, and the frequency of the mutant genotype (TT) was 40.9%. We found an association between the presence of stent restenosis and the presence of the -786T>C variant: in the patients with and without restenosis, the frequency of the CC genotype was 24.2% and 51.7%, the frequency of the TC genotype was 12.1% and 20.0%, and the frequency of the TT genotype was 63.6% and 28.3%, respectively (P = 0.023). In the multivariate logistic regression analysis, along with the presence of the -786T>C variant, the other determinants of stent restenosis included male gender, waist circumference, both systolic and diastolic blood pressures, history of dyslipidemia, left anterior descending artery (LAD) involvement, distal position of stenting, and duration of the concomitant use of aspirin and Plavix®. However, in similar analysis, none of the pointed factors could predict the severity and percentage of restenosis.

Conclusion: The presence of the -786T>C polymorphism of the eNOS gene is a major and serious risk factor for stent restenosis, independent of the effects of other cardiovascular risk factors. The effect of this polymorphism is particularly highlighted in the LAD. Nevertheless, it seems that the -786T>C polymorphism may not have a central role in the progression and severity of stent restenosis.***(Iranian Heart Journal 2015; 16(2):35-40)***

Keywords: -786T>C ■ Endothelial Nitric Oxide Synthase ■ In-Stent Restenosis ■ Coronary Stent ■ Percutaneous Coronary Intervention ■ Polymorphism

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Coronary stents, which were first developed in the mid-1980s, have ultimately replaced the old balloon angioplasty as the preferred method of performing percutaneous coronary interventions (PCI).[2] After the observed improvements in angiographic and clinical outcomes, this field represents the most common major medical procedure worldwide. Despite the high successful rates of this procedure, stent restenosis remains a frequent complication following coronary stenting. The most important risk factors of modulator intimal hyperplasia and restenosis are ischemia/reperfusion injury, shear stress, inflammation, diabetes, oxidative stress, hypertension, modulation of cytokine, and C-reactive protein (CRP) level, together with other environmental stimuli such as smoking. In this regard, platelet aggregation, inflammatory cell infiltration, release of growth factors, medial smooth muscle cell modulation and proliferation, proteoglycan deposition, and extracellular matrix remodeling have been identified as the major milestones for creating background for stent restenosis. In total, stent restenosis is a multifactorial process due to a complex endothelial and inflammatory response to the stent deployment. Alongside environmental factors, some observations have suggested some gene polymorphisms predisposing individuals to stent restenosis. This observation has led several investigators to propose and study several genes, and a number of genetic polymorphisms have been identified that affect the molecules likely to be involved in the pathophysiology of restenosis after coronary stenting. In this regard, the role of polymorphisms on the sequences of endothelial nitric oxide synthase (eNOS) has been proposed. Some evidence is currently available on the relationship between the -786T>C variant of the eNOS gene and sensitivity to stent restenosis. However, in a study by Gomma et al,[8] only a significant impact of this variant of the eNOS gene on stent restenosis was revealed and, thus, highlighted the importance of the nitric oxide (NO) system in preventing in-stent restenosis. We conducted the present study to probe into the possible involvement of the role of the -786T>C variant of the eNOS gene in stent restenosis following coronary stent deployment.

Methods

This cross-sectional study was conducted on 100 consecutive patients who underwent coronary stenting because of significant coronary artery disease at Shariati Hospital in 2014. This population was assigned to a case group, who had restenosis and were candidated for revascularization (n, 50), and a matched control group, who underwent coronary stenting but without evidence of restenosis within 6 months of stenting (n, 50). The information on stent restenosis was obtained by reviewing the coronary angiography documents. The exclusion criteria were the existence of active infection, presence of neoplastic or inflammatory evidence, acute myocardial infarction during the preceding 4 weeks, history of percutaneous coronary interventions in the same coronary artery, or history of renal or hepatic failure. The study protocol was approved by our institutional ethics committee, and all the patients gave written informed consent for the intervention. Baseline characteristics were collected by reviewing the hospital records. The presence

of the gene polymorphism was assessed as follows: First, DNA was extracted from whole blood samples using the salting out method and was stored at 20 °C in 2 mL screw-cap Apex tubes. The samples were diluted 1 in 20 in sterile deionized water for a working array using 96-deep well microplates and stored at 4 °C. The -786T>C polymorphism was identified, following polymerase chain reaction (PCR), via restriction enzyme digestion by Mspl enzyme. All the statistical analyses were performed using Statistical Packages for the Social Sciences (SPSS), version 20.0. The χ^2 test was used to compare the nominal variables between the groups, and the *t*-test or the Mann-Whitney U test was used for the numerical variables. A *P* value < 0.05 was considered statistically significant.

Results

After excluding the incomplete files, 93 patients were assessed. The mean age of the participants was 61.23 ± 7.35 years (range, 45 to 80 years), and 62.4% were male. The mean body mass index was 26.90 ± 3.00 kg/m^2, and the mean waist circumference was 91.66 ± 10.87 cm. Regarding cardiovascular risk factors, 39.8% of the patients were diabetic, 47.3% had dyslipidemia, 55.9% were hypertensive, 35.5% were smokers, 53.8% had a family history of cardiovascular disease, 10.8% were anemic, and 8.6% had a history of renal failure. The mean left ventricular ejection fraction (LVEF) was 47.01 ± 7.74%. Except for 1 stent, the others were drug-eluting (98.9%). As regards the stent point, 46.2% were implanted in the left anterior descending artery (LAD), 19.4% in the left circumflex artery (LCX), and 34.4% in the right coronary artery (RCA). The stents were implanted proximally in 50.5%, medially in 43.0%, and distally in 6.5%. The mean duration of stent implantation was 3.17 ± 1.46 years. Also, the mean duration of the concurrent use of aspirin and Plavix® was 0.94 ± 0.14 years. The overall prevalence of restenosis was 34.4%. In stents with restenosis condition, the mean percentage of stenosis was 71.56 ± 14.56%. In total, the reason for recent angiography was chronic stable angina in 48.4% and acute coronary syndrome in 51.6%.

The frequency of the wild genotype (CC) of the -786T>C variant was 41.9%, the frequency of the heterozygous genotype (TC) was 41.9%, and the frequency of the mutant genotype (TT) was 40.9%. Apropos the frequency of the -786T>C variant in different patient subgroups, there were no significant differences in the frequency of the -786T>C genotype between the men and the women (*P* = 0.589), between the diabetic and non-diabetic patients (*P* = 0.368), between the patients with and without dyslipidemia (*P* = 0.967), between the hypertensive and normotensive patients (*P* = 0.399), between the smokers and nonsmokers (*P* = 0.439), and between the patients with and without a family history of coronary disease (*P* = 0.580). Also, the presence of the -786T>C variant was not associated with the patients' age (*P* = 0.941), body mass index (*P* =0.395), systolic blood pressure (*P* = 0.854), diastolic blood pressure (*P* = 0.977), and LVEF (*P* = 0.245).

There was a significant relation between the type of the stent-implanted coronary artery and the genotypes of the -786T>C variant, such that in each of the LAD, LCX, and RCA, the frequency of the CC genotype was 30.2%, 38.9%, and 59.4%; the frequency of the TC genotype was 11.6%, 16.7%, and 25.0%; and the frequency of the TT genotype was 58.1%, 44.4%, and 15.6%, respectively (*P* = 0.008). There was no relation between the -786T>C polymorphisms and the position of stent implantation, such that in the proximal, medial and distal positions, the frequency of the CC genotype was 48.9%, 32.5%, and 50.0%; the frequency of the TC genotype was 12.8%, 20.0%, and 33.3%; and the frequency of the TT genotype was 38.3%, 47.5%, and 16.7%, correspondingly (*P* = 0.344). Our results also demonstrated an association

between the presence of stent restenosis and the presence of the -786T>C variant, such that in the patients with and without restenosis, the frequency of the CC genotype was 24.2% and 51.7%; the frequency of the TC genotype was 12.1% and 20.0%; and the frequency of the TT genotype was 63.6% and 28.3%, correspondingly (P = 0.023). In the multivariate logistic regression analysis (Table 1), in conjunction with the presence of the -786T>C variant, the other determinants of stent restenosis included male gender, waist circumference, both systolic and diastolic blood pressures, history of dyslipidemia, LAD involvement, distal position of stenting, and duration of the concomitant use of aspirin and Plavix®. However, in a similar analysis (Table 2), none of the pointed factors could predict the severity and percentage of restenosis.

Table 1. Main Determinants of the Presence of Stent Restenosis

	B	SE	Wald	*df*	Sig.	Exp(B)
Genotype	-.874	0.443	3.900	1	0.048	0.417
Sex	2.463	1.019	5.848	1	0.016	11.741
Age	0.074	0.057	1.699	1	0.192	1.077
BMI	0.000	0.131	0.000	1	0.995	0.999
WC	0.095	0.047	4.090	1	0.043	1.099
SBP	0.105	0.055	3.689	1	0.055	1.111
DBP	-0.150	0.065	5.376	1	0.020	0.861
Renal	-0.515	1.224	0.177	1	0.674	0.597
DM	0.699	0.890	0.616	1	0.432	2.011
DL	2.493	0.807	9.552	1	0.002	12.096
HTN	1.704	1.343	1.609	1	0.205	5.494
CS	-0.097	0.825	0.014	1	0.906	0.908
FH	1.140	0.817	1.945	1	0.163	3.125
Anemia	0.328	1.261	0.068	1	0.795	1.388
EF	-0.018	0.061	0.082	1	0.774	0.983
Vessel			4.306	2	0.116	
Vessel(1)	-1.977	0.966	4.186	1	0.041	0.138
Vessel(2)	-1.801	1.259	2.047	1	0.153	0.165
v5			4.058	2	0.131	
v5(1)	-0.883	1.440	0.376	1	0.539	0.413
v5(2)	-1.816	0.922	3.875	1	0.049	0.163
Date	-0.006	0.258	0.001	1	0.982	0.994
Time	6.739	3.270	4.246	1	0.039	844.316
Constant	-27.395	11.198	5.985	1	0.014	0.000

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; DM, diabetes mellitus; WC, waist circumference; DL, dyslipidemia; CS, cigarette smoking; FH, family history; HTN, hypertension; EF, ejection fraction.

Table 2. Main Determinants of the Severity and Percentage of Stent Restenosis

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.
		B	Std. Error	Beta		
1	(Constant)	118.073	70.811		1.667	0.107
	Sex	-8.267	10.951	0.215	-0.755	0.457
	Age (y)	-0.342	0.600	-0.111	-0.570	0.574
	BMI	0.856	1.252	0.169	0.683	0.501
	WC	-0.028	0.452	-0.017	-0.061	0.952
	SBP	-0.321	0.301	-0.397	-1.066	0.296
	DBP	0.629	0.505	0.426	1.247	0.224
	Renal	1.937	9.954	0.038	0.195	0.847
	DM	6.245	7.884	0.174	0.792	0.435
	DL	-7.753	6.846	-0.218	-1.133	0.268
	HTN	1.405	11.362	0.037	0.124	0.903
	CS	0.072	8.919	0.002	0.008	0.994
	FH	-9.346	7.505	-0.264	-1.245	0.224
	Anemia	-8.598	11.908	-0.141	-0.722	0.477
	EF	-0.071	0.525	-0.028	-0.136	0.893
	Date	-1.168	2.613	-0.086	-0.447	0.659
	Time	-19.829	18.355	-0.202	-1.080	0.290

[a.] Dependent variable: percent

Abbreviations: BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; DM, diabetes mellitus; DL, dyslipidemia; HTN, hypertension; FH, family history; EF, ejection fraction.

Discussion

Chiming in with previous research on the role of the eNOS gene polymorphisms, especially the -786T>C variant—in stent restenosis—we aimed to assess the value of this polymorphism to predict stent restenosis in the Iranian population. In addition to the role of this variant in the assessment of both presence and severity of stenosis, we also determined other indicators of stent restenosis. First, in line with previous studies, the present study showed an association between the presence of stent restenosis and the presence of the -786T>C variant. Second, according to our results, it seems that the presence of this variant was mainly related to restenosis in the stents implanted in the LAD. However, the presence of this polymorphism could not predict the severity of restenosis. Interestingly, the effect of this variant on stent restenosis was independent of other cardiovascular risk factors. Our result was in agreement with other similar studies. In a study by Gomma et al[8] on 226 patients who underwent elective and successful coronary artery stenting to de novo lesions in native coronary arteries, the carriers of the 298Asp allele of the eNOS Glu298Asp polymorphism showed a higher frequency of restenosis. In addition, the carriers of the -786C allele of the eNOS -786T>C polymorphism also showed a higher frequency of restenosis with an odds ratio of 2.06. These effects were essentially additive and were independent of other classical risk factors.

As has been previously shown, nitric oxide originating from the endothelium is a main atheroprotective mediator and, thus, an abnormality in its regulation has been established as an important cardiovascular factor.[9-11] It has been shown that the deletion occurring in the eNOS gene in animal models leads to a rise in blood pressure and an increase in predisposition to coronary atherosclerosis.[12] Various variants in the eNOS gene have been identified to be associated with cardiovascular pathological conditions. One of the most important variants is the -786T>C polymorphism. Accordingly, it can be concluded that not only can this polymorphism have a major role in promoting atherosclerosis, but also it can effectively predict stent restenosis.

Conclusion

The presence of the -786T>C polymorphism of the eNOS gene is a major and serious risk factor for stent restenosis, independent of the effects of other cardiovascular risk factors. The effect of this polymorphism is particularly highlighted in the LAD. However, it seems that the -786T>C polymorphism may not have a central role in the progression and severity of stent restenosis.

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