ORIGINAL ARTICLE

Role of polymorphisms of the endothelial nitric oxide synthase gene in predicting slow-flow phenomenon after primary percutaneous coronary intervention

Primer perkütan koroner girişim sonrası yavaş akım fenomeninin öngörülmesinde endotelyal nitrik oksit sentaz geni polimorfizmlerinin rolü

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ABSTRACT

Objective: The aim of the present study was to examine the association between 2 polymorphisms of the endothelial nitric oxide (eNOS) gene (-786T>C and +894G>T) and the no-reflow/slow-flow phenomenon in post-primary percutaneous coronary intervention (PPCI) patients.

Methods: A total of 103 post-PPCI patients were enrolled. Coronary no-reflow phenomenon was defined as a Thrombolysis in Myocardial Infarction (TIMI) flow grade 0–1 and coronary slow-flow phenomenon (CSFP) was defined as a TIMI flow grade ≤2.

Results: Due to the small number of post-PPCI patients with the no-reflow phenomenon (n=4), the primary comparison was made between CSFP (n=20) and normal flow (n=83) groups. There was a greater frequency of CSFP among carriers of the –786C allele of the eNOS –786T>C polymorphism (odds ratio [OR]: 3.90; 95% confidence interval [CI]: 0.87–17.45; p=0.07). However, no such association was detected between the +894T allele of the eNOS +894G>T and CSFP (OR: 0.92; 95% CI: 0.21–3.98; p=0.91). In the adjusted analysis, the -786T>C polymorphism did not reach statistical significance.

Conclusion: There was no significant association between CSFP and 2 of the most common polymorphisms of the eNOS gene in post-PPCI patients.

ÖZET

Amaç: Bu çalışmada, primer perkütan koroner girişim (PPKG) yapılan hastalarda endotelyal nitrik oksit (eNOS) geninin (-786T>C ve +894G>T) iki polimorfizmi ile akımsızlık /yavaş akım fenomeninin ilişkisini araştırmayı amaçladık.

Yöntemler: PPKG sonrası toplam 103 hasta çalışmaya dahil edildi. Koroner akımsızlık fenomeni miyokart enfarktüsünde tromboliz (TIMI) akım derecesi 0–1, koroner yavaş akım fenomeni (KYAF) ise TIMI akım derecesi ≤2 olarak tanımlandı.

Bulgular: PPKG sonrasında akımsızlık gelişen hasta sayısının azlığı (n=4) nedeniyle, ana karşılaştırma KYAF (n=20) ve normal akım (n=83) grupları arasında yapıldı. eNOS –786T>C polimorfizminin –786C aleli taşıyıcıları, daha yüksek sıklıkta KYAF'a sahip olma eğilimi gösterdi (OR: 3.90, %95 GA: 0.87–17.45; p=0.07). Bununla birlikte, eNOS +894G>T ve KYAF'nin +894T aleli için böyle bir ilişki saptanmadı (OR: 0.92, %95 GA: 0.21–3.98; p=0.91). Düzeltilmiş analizde, ayarlamalar yapıldıktan sonra -786T> C polimorfizmi istatistiksel öneme ulaşmadı.

Sonuç: PPKG sonrası hastalarımızda KYAF ile eNOS geninin en sık görülen polimorfizmlerinden ikisi arasında anlamlı bir ilişki bulamadık.

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Acute ST-segment elevation myocardial infarction (STEMI) is most often caused by the thrombotic occlusion of an epicardial coronary artery.[1–3] Primary percutaneous coronary intervention (PPCI) is currently the preferred treatment strategy for patients with STEMI in that it can successfully reopen the occluded vessel in more than 95% of cases.[1] Interestingly, even when the mechanical obstruction is corrected, the flow will not be adequately restored in a significant number of patients. This condition, termed no-reflow phenomenon, may occur in up to 40% of post-PPCI patients. No-reflow phenomenon is considered a significant independent predictor of in-hospital mortality, major adverse cardiac events, and malignant arrhythmias, and can, thus, lessen the beneficial impact of PPCI.[1 3–6]

Given the abovementioned important prognostic effects of the no-reflow phenomenon, multiple investigations have tried to determine potential predictors. Demographic characteristics (e.g., age and gender),[7] clinical presentation,[8–11] the presence of traditional coronary risk factors, laboratory findings (e.g., hyperglycemia[12] and hematological indices[13]), angiographic properties (e.g., thrombus grade[14] and the SYNTAX score[15]), and procedural factors (e.g., the use of post-dilation[16] and long stenting[17]) have all been considered as potential candidates for predicting the risk of diminished flow after PPCI.

In addition, attempts have been made to find molecular or genetic predictors of the pathophysiology of STEMI. The pathophysiology of the no-reflow phenomenon is complex and multifactorial insofar as such mechanisms as platelet activation, distal embolization, coronary spasm, reperfusion injury, and localized inflammation lead to endothelial dysfunction,[17–19] and hence, diminish vasodilation and create microvascular flow impairment.[20,21] Normally, the healthy endothelium regulates vascular tone via the release of nitric oxide (NO).[22] NO serves to relax the vascular smooth muscle, to inhibit platelet activation and leukocyte adhesion, and to modulate the migration and growth of vascular smooth muscle cells.[4,5] Consequently, alterations in the NO pathway and its enzyme, endothelial nitric oxide synthase (eNOS), may cause endothelial dysfunction.[5]

Several polymorphisms have been discovered in the eNOS gene. Two common variants, a variant with a T/C substitution in the 5′ flanking region near the promoter at position –786 and a variant with a G/T substitution at position 894 in exon 7 that codes for the replacement of glutamic acid with aspartic acid, have been linked by several groups of investigators to the risk of coronary spasm, coronary artery disease (CAD), and acute myocardial infarction (MI).[22–25] Therefore, functionally important polymorphisms of the eNOS gene might be related to individual differences in susceptibility to ischemic injury during STEMI and explain the diverse prevalence of the no-reflow phenomenon.[5]

Abbreviations:

CAD	*Coronary artery disease*
CI	*Confidence interval*
CSFP	*Coronary slow-flow phenomenon*
eNOS	*Endothelial nitric oxide*
MI	*Myocardial infarction*
NF	*Normal flow*
NO	*Nitric oxide*
OR	*Odds ratio*
PCR	*Polymerase chain reaction*
PPCI	*Primary percutaneous coronary intervention*
RFLP	*Restriction fragment length polymorphism*
SNP	*Single-nucleotide polymorphism*
STEMI	*ST-segment elevation myocardial infarction*
TIMI	*Thrombolysis in Myocardial Infarction*

The aim of the present study was to demonstrate the correlation between the occurrence of the no-reflow/slow-flow phenomenon in post-PPCI patients and polymorphisms of the eNOS gene and to compare it with other suggested predisposing factors.

METHODS

Study design

Between August 2017 and September 2018, all patients with acute STEMI who were candidates for PPCI at Rajaie Cardiovascular Medical and Research Center were enrolled in this single-center, prospective, cross-sectional study. The diagnosis of STEMI was made according to the Third Universal Definition of Myocardial Infarction.[26] The exclusion criteria were age <18 years, allergy to the contrast agent, unsuccessful PPCI, and previous revascularization (i.e., coronary bypass graft surgery or PCI). Written informed consent was obtained from all the patients before blood sampling, and the study protocol was approved by the Ethics Committee of Rajaie Cardiovascular Medical and Research Center (ethics approval number: 94015).

Blood samples, collected just before the start of the PCI procedure, were used to analyze biochemical parameters, hematological indices, and cardiac

biomarkers through standard methods. For the genetic analysis, 2 cc of each sample was kept at -20°C. Fasting blood glucose levels and lipid profiles were recorded using a sample collected on the postprocedural day.

All of the patients had received combined antiplatelet therapy with loading doses of 325 mg of aspirin and 600 mg of clopidogrel at the time of the diagnosis of STEMI. PPCI was performed according to the latest standard guidelines.[27] At the beginning of the procedure, a weight-based loading dose of unfractionated heparin was injected intravenously and complementary doses were administered according to the activated clotting time during the procedure. The use of glycoprotein IIb/IIIa inhibitors, aspiration thrombectomy, and pre- or post-dilation was at the discretion of the operator.

The coronary angiograms were evaluated by 2 experienced interventional cardiologists, who were blinded to the patients' clinical data. The coronary blood flow patterns were assessed based on the Thrombolysis in Myocardial Infarction (TIMI) flow grade, immediately before and after PPCI. The TIMI grade was classified as follows: grade 0, no perfusion; grade 1, presentation without perfusion; grade 2, partial perfusion; and grade 3, complete perfusion.[28] No-reflow phenomenon was defined as a TIMI flow grade 0–1, and coronary slow-flow phenomenon (CSFP) was defined as a TIMI flow grade ≤2.[29] Mechanical complications, such as coronary dissection and spasm, were excluded from the analysis. The SYNTAX score was calculated using the online calculator (http://www.syntaxscore.com). Thereafter, the patients were categorized into tertiles based on the calculated SYNTAX scores: low (<23), intermediate,[23–32] and high (>32).[30]

The thrombus grade was evaluated using the TIMI thrombus scale: grade 0, no angiographic sign of thrombi was detected; grade 1, possible angiographic characteristics of thrombi, such as decreased contrast density, haziness, and irregular lesion contours observed; grade 2, a definite thrombus ≤1/2 the vessel diameter; grade 3, the largest dimension of the thrombus was >1/2 but <2 the vessel diameter; grade 4, the thrombus was >2 the vessel diameter; grade 5, total occlusion.[31] The TIMI thrombus grades were categorized as high (grades 4 and 5) and low thrombus grades (grades 1–3).

The CHA2DS2-VASc score was calculated according to the points assigned to each of the risk predictors: congestive heart failure (1 point); hypertension (1 point); age ≥75 years (2 points); diabetes mellitus (1 point); previous stroke, transient ischemic attack, or thromboembolism (2 points); vascular diseases (i.e., history of MI, peripheral arterial disease, or complex aortic plaques) (1 point); age between 65 and 74 years (1 point); and female gender (1 point).

Determination of the polymorphisms of the eNOS gene

For the purposes of DNA extraction, 200 μL of blood from each of a total of 119 patients was collected in tubes containing pre ethylenediaminetetraacetic acid (0.4 M at pH 8.0). The GF-1 Blood DNA Extraction Kit (Vivantis Technologies, Shah Alam, Selangor Darul Ehsan, Malaysia) was used, and genomic DNA was isolated according to the company's manual. The 894G>T and -786T>C mutations in the eNOS gene were analyzed using polymerase chain reaction (PCR), followed by restriction fragment length polymorphism (RFLP). The amplification of 894G>T was completed using forward primer 5'-TCCCTGAGGAGGGCATGAGGCT-3' and reverse primer 5'-TGAGGGTCACACAGGTTCCT -3'.[32] The amplification of -786T>C was performed using forward primer 5'-AGTTTCCCTAGTCCCCCATGC-3' and reverse primer 5'-CCACACCCCCATGACTCAAGT-3'.[33] PCR was performed in a total volume of 30 μL containing 100 ng of genomic DNA, 25 mM of magnesium chloride, 500 μM of each of deoxynucleoside triphosphates, and 1 U of Taq DNA polymerase. With respect to the PCR conditions, the amplification of 894G>T began with an initial denaturation at 95°C for 1 minute, 35 cycles at 94°C for 1 minute, 61°C for 1 minute (annealing), and 72°C for 1 minute, followed by a final extension at 72°C for 7 minutes. The amplification of -786T>C was performed with an initial denaturation at 94°C for 1 minute, 30 cycles at 94°C for 45 seconds, 61°C for 1 minute (annealing), and 72°C for 1 minute, followed by a final extension at 72°C for 7 minutes. The Ban II restriction enzyme (New England Biolabs, Inc., Ipswich, MA, USA) was used at 37°C for 20 hours for 894G>T, while the NgoMIV restriction enzyme (New England Biolabs, Inc., Ipswich, MA, USA) was used at 37°C for 2 hours for -786T>C. The PCR and RFLP products were visualized on 12.5% polyacrylamide electrophoresed gel.

Statistical analysis

Deviations from the Hardy-Weinberg equilibrium for each single-nucleotide polymorphism (SNP) were determined using chi-square analysis with 1 degree of freedom. The sample size was calculated according to a previous study published by our center.[8] The quantitative data were described as the mean±SD for normally distributed data based on the Shapiro-Wilk test, while the non-normal variables were presented as the median (interquartile ranges: P25–P75). The between-group differences were analyzed using the Student's t-test and the Mann-Whitney U test, where indicated. The categorical data were expressed as percentages and were tested using a chi-square test and the Fisher exact test was used in the event of small expected cell counts.

Binary logistic regression was employed to determine the effects of various factors on CSFP. The selection process of the variables in the multivariate logistic modeling was performed in accordance with the Hosmer-Lemeshow guideline.[34] The odds ratio (OR) of the adjusted predictors for CSFP in 894G>T was not calculated because of the higher p value of unadjusted 894G>T.

All p values were 2-tailed, and a p value <0.05 was considered statistically significant. The statistical analyses were performed using Stata version 14 software (StataCorp LLC, College Station, TX, USA).

RESULTS

Between August 2017 and September 2018, the present study recruited 119 patients, of whom 16 individuals were excluded due to inadequate DNA extraction. Due to the small number of patients with the no-reflow phenomenon, the primary analysis was conducted between patients with CSFP (TIMI flow grade ≤2) and those with normal flow (NF) after PPCI. The demographic and clinical characteristics of the study population are depicted in Table 1. There were no significant differences between the groups in terms of age, gender, or diabetes mellitus. A history of diabetes mellitus was present in 21.4% of the study population.

In terms of the traditional coronary risk factors, there were no statistically significant differences between the CSFP and NF groups concerning hypertension or a family history of CAD (Table 1). Cigarette smoking was significantly less frequent in the CSFP group than in the NF group (30.0% vs 54.2%; p=0.05).

Determinants of the patients' clinical presentation (i.e., blood pressure and heart rate on admission, duration of chest pain, and Killip class) were fairly similar between the 2 groups (Table 1).

The level of blood sugar on admission in the CSFP group was significantly higher than that of the NF group (median: 200.00 mg/dL [min-max: 136.00–293.00 mg/dL] vs median: 137.00 mg/dL [min-max: 116.50–170.50 mg/dL]; p=0.02). All hematological indices studied in previous reports (i.e., white blood cell, neutrophil, lymphocyte, and platelet counts together with the lymphocyte/platelet ratio) were analyzed in the present study as well: The results demonstrated that none was statistically significantly different between the 2 study groups except hemoglobin (mean: 13.70±1.50 g/dL in the CSFP group vs mean: 14.40±1.50 g/dL in the NF group; p=0.04). The platelet count was marginally higher in the CSFP group than in the NF group (median: 250,000.00 [min-max: 198,500.00–282,000.00] vs median: 226,500.00 [min-max: 204,000.00–249,250.00]; p=0.07).

The angiographic characteristics of the patients are summarized in Table 2, which shows that 43 (41.7%) patients had single-vessel disease, 35 (33.9%) double-vessel disease, and 25 (24.3%) triple-vessel disease. The culprit artery was the left anterior descending in 47 (45.6%) patients, the right coronary artery in 34 (33.0%), and the left circumflex artery in 6 (5.8%). Additionally, 16 (15.5%) patients had thrombotic lesions in the obtuse marginal, diagonal branches, or the ramus intermedius. No clear difference was detected between the 2 groups regarding vessel involvement. The mid-portion of the vessel was the most commonly affected site (53 [51.5%] patients). The proportion of the distal involvement of the coronary vasculature in the CSFP group was significantly higher than that of the NF group (20.0% vs 4.8%; p=0.03). The thrombus grade and high thrombus grade (thrombus grade >3) findings during the diagnostic angiography in the culprit vessel were similar between the 2 study groups.

The mean SYNTAX score was 16.67±6.74 in the CSFP group and 15.90±8.27 in the NF group. Neither the SYNTAX score nor the SYNTAX category was significantly different between the 2 groups.

Among the procedural factors (Table 3), the mean door-to-balloon time was similar between the CSFP and NF groups (median: 27.50 minutes [min-max:

21.25–33.75 minutes] vs median: 25.00 minutes [min-max: 20.00–30.00 minutes]; p=0.51). There were no statistically significant differences between the 2 groups with respect to the stent length (median: 29.00 mm [min-max: 18.50–35.25 mm] vs median: 24.00 mm [min-max: 18.00–30.00 mm]; p=0.14), or the stent diameter (median: 3.00 mm [min-max: 2.75–3.37] vs median: 3.00 [min-max: 2.75–3.50]; p=0.23).

As previously explained, the use of pre- and post-dilation was left to the operator's discretion: 41 (39.8%) patients had pre-dilation and 13 (12.7%) post-dilation. Neither was related to the post-PCI TIMI flow.

Table 1. Baseline demographic, clinical, and laboratory characteristics of the patients

Variable	Total sample (n=103)	Normal-flow group (n=83)	Slow-flow group (n=20)	Normal flow vs slow flow *p* value
Age (years)	58.14±12.37	57.14±12.23	62.30±12.38	0.09
Female	17 (16.5)	12 (14.5)	5 (25.0)	0.26
Diabetes mellitus	22 (21.4)	16 (19.3)	6 (30.0)	0.29
Traditional risk factors				
Hypertension	38 (36.9)	30 (36.1)	8 (40.0)	0.74
Cigarette smoking	51 (49.5)	45 (54.2)	6 (30.0)	0.05
Family history	16 (15.5)	13 (15.7)	3 (15.0)	0.90
Clinical state on arrival				
Blood pressure	130.18±29.68	131.44±31.10	124.95±22.80	0.47
Heart rate	80.70±17.27	81.06±17.04	79.25±18.61	0.67
Killip II class at admission >1	17 (16.5)	12 (14.5)	5 (25.0)	0.25
Chest pain duration (hours)	3.00 (2.00–5.00)	3.00 (2.00–5.00)	4.00 (2.50–4.75)	0.52
CHA2DS2-VASc score >2	49 (47.6)	37 (44.6)	12 (60.0)	0.21
CHA2DS2-VASc score	1.00 (1.00–2.00)	1.00 (1.00–2.00)	2.00 (1.00–2.00)	0.37
Left ventricular ejection fraction	37.08±9.67	37.01±9.65	37.36±10.05	0.88
Blood sugar on admission	142.00 (117.25–199.00)	137.00 (116.50–170.50)	200.00 (136.00–293.00)	0.02
Fasting blood sugar (mg/dL)	119.00 (105.00–156.5.00)	118.50 (104.25–155.50)	122.50 (106.50–158.25)	0.64
Hemoglobin (g/dL)	14.21±1.98	14.45±1.50	13.21±3.17	0.04
Total cholesterol (mg/dL)	171.23±41.15	171.32±39.64	170.80±49.51	0.96
Low-density lipoprotein (mg/dL)	108.00 (78.00–127.00)	108.50 (79.50–125.25)	92.00 (65.00–130.00)	0.70
Triglyceride (mg/dL)	127.00 (88.00–171.5)	128.50 (86.75–170)	127 (94–215)	0.77
High-density lipoprotein (mg/dL)	38.00 (35.00–45.00)	37.50 (34.50–45.00)	39.00 (36.00–47.00)	0.43
Cr (mg/dL)	0.90 (0.80–1.00)	0.90 (0.80–1.00)	0.90 (0.80–1.22)	0.71
Blood urea nitrogen (mg/dL)	15.00 (12.50–20.00)	15.00 (12.50–19.00)	15.50 (12.25–21.00)	0.79
White blood cell	10600.00 (8775.00–10600.00)	10600.00 (8775.00–12425.00)	10750.00 (8725.00–13007.50)	0.76
Neutrophilic count	8136.28±2841.15	8080.23±2663.24	8366.07±3550.04	0.68
Platelet count	232500.00 (204000.00–261250.00)	226500.00 (204000.00–249250.00)	250000.00 (198500.00–282000.00)	0.076
Platelet/lymphocyte	12834.82 (9215.27–18484.61)	12834.82 (9018.29–18316.66)	12903.51 (9354.16–22123.62)	0.50

Data are presented as number (%), mean±SD or median (minimum-maximum).

Table 2. Angiographic characteristics of the patients

Angiographic characteristic	Total sample (n=103)	Normal-flow group (n=83)	Slow-flow group (n=20)	*p* value
Number of diseased vessels (%)				
Single-vessel disease	43 (41.7)	35 (42.2)	8 (40)	0.43
Two-vessel disease	35 (33.9)	26 (31.3)	9 (45)	
Three-vessel disease	25 (24.3)	22 (26.4)	3 (15)	
Culprit (%)				
Left anterior descending artery	47 (45.6)	38 (45.8)	9 (45)	0.90
Left circumflex coronary artery	6 (5.8)	5 (6)	1 (5)	
Right coronary artery	34 (33)	27 (32.5)	7 (35)	
Obtuse marginal, diagonal branches, ramus intermedius, posterior left ventricle	16 (15.5)	13 (15.6)	3 (15)	
Location (%)				
Proximal	42 (40.8)	37 (44.6)	5 (25)	0.03
Mid-portion	53 (51.5)	42 (50.6)	11 (55)	
Distal	8 (7.8)	4 (4.8)	4 (20)	
Thrombus grade (%)				
≤3	18 (17.5)	14 (16.9)	4 (20)	0.74
4 and 5	85 (82.5)	69 (83.1)	16 (80)	
SYNTAX score (mean±SD)	16.05±7.97	15.90±8.27	16.67±6.74	0.45
SYNTAX category (%)				
Low	81 (78.6)	66 (79.5)	15 (75)	0.65
Intermediate	19 (18.4)	14 (16.9)	5 (25)	
High	3 (2.9)	3 (3.6)	0 (0)	

Both SNPs (-786T>C and 894G>T) satisfied the Hardy-Weinberg equilibrium.

The genotype frequency (Table 4) of the -786T>C polymorphism for T/T, T/C, and C/C was 47 (45.6%), 45 (43.7%), and 11 (10.7%), respectively, in the entire study population. There was no statistically significant relationship between the groups regarding the -786T>C genotype. The patients with the T/T genotype were regarded as the reference group (Table 5). In comparison with the T/T group, the OR of CSFP was 1.95 for patients with T/C (95% CI: 0.64–5.91; p=0.23) and 3.90 for patients with C/C (95% CI: 0.8–17.45; p=0.07) (Table 5).

The genotype distribution (Table 4) of the 894G>T polymorphism for G/G, G/T, and T/T was 44 (42.7%), 45 (43.7%), 14 (13.6%), respectively, in the entire study population. As in the -786T>C variant, there was no statistically significant relationship between the groups concerning the genotypes of the 894G>T polymorphism (Table 4). In this polymorphism, the patients with G/G were considered the reference group (Table 5). In comparison with the G/G group, the OR of CSFP was 0.92 for patients with T/T, which was nonsignificant (95% CI: 0.21–3.98; p=0.91), and the OR of CSFP for patients with 894G>T was 0.62, which did not constitute statistical significance (95% CI: 0.21–1.82; p=0.39). As was previously clarified, the adjusted analysis was performed only for -786T>C due to the higher p value of unadjusted 894G>T. After adjustments, -786T>C was not a significant predictor of CSFP following PPCI (Table 5).

Table 6 reports the adjusted and unadjusted effects of potential clinical predictors of CSFP after PPCI. In multivariate analysis, the platelet count, the blood sugar level on admission, and the hemoglobin level failed to reach statistical significance after adjustments.

DISCUSSION

In the present study, we sought to investigate the value of the polymorphisms of the eNOS gene in predicting occurrence of the no-reflow phenomenon following PPCI. We also compared prognostic efficacy of these gene polymorphisms and other previously suggested predisposing factors. Although multiple studies have

Table 3. Procedural characteristics of the patients

Procedural characteristic of PCI	Total sample (n=103)	Normal-flow group (n=83)	Slow-flow group (n=20)	*p* value
Door-to-balloon time (minutes)	25.00 (20.00–30.00)	25.00 (20.00–30.00)	27.50 (21.25–33.75)	0.51
Stent length (mm)	25.00 (18.00–32.00)	24.00 (18.00–30.00)	29.00 (18.50–35.25)	0.14
Stent diameter (mm)	3.00 (2.75–3.50)	3.00 (2.75–3.50)	3.00 (2.75–3.37)	0.23
Pre-dilation	41 (39.8)	31 (37.3)	10 (50)	0.30
Post-dilation	13 (12.7)	13 (15.9)	0 (0)	0.05
Pre-PCI TIMI flow				
TIMI 0	82 (79.6)	67 (80.7)	15 (75)	0.78
TIMI 1	7 (6.8)	5 (6)	2 (10)	
TIMI 2	3 (2.9)	2 (2.4)	1 (5)	
TIMI 3	11 (10.7)	9 (10.8)	2 (10)	
Post-PCI TIMI flow				
TIMI 1	4 (3.9)		4 (20)	<0.001
TIMI 2	16 (15.5)		16 (80)	
TIMI 3	83 (80.6)	83 (100)		
TIMI<3	103 (100)		20 (100)	

PCI: Percutaneous coronary intervention; TIMI: Thrombolysis in Myocardial Infarction.

Table 4. Genotype and allele frequency of +894G>T and -786T>C polymorphisms of the eNOS gene

	Total sample (n=103)	Normal-flow group (n=83)	Slow-flow group (n=20)	*p* value
+894G>T polymorphism, n (%)				
G/G	44 (42.7)	34 (41)	10 (50)	0.67
G/T	45 (43.7)	38 (45.8)	7 (35)	
T/T	14 (13.6)	11 (13.3)	3 (15)	
Allele				
G	133 (64.6)	106 (63.85)	27 (67.5)	0.46
T	73 (35.4)	60 (36.14)	13 (32.5)	
-786T>C polymorphism, n (%)				
T/T	47 (45.6)	41 (49.4)	6 (30)	0.16
T/C	45 (43.7)	35 (42.2)	10 (50)	
C/C	11 (10.7)	7 (8.4)	4 (20)	
Allele, n (%)				
T	139 (67.5)	117 (70.48)	22 (55)	0.11
C	67 (32.5)	49 (29.51)	18 (45)	

eNOS: Endothelial nitric oxide synthase.

Table 5. Adjusted and unadjusted effects of the +894G>T and -786T>C polymorphisms of the eNOS gene as predictors of the coronary slow-flow phenomenon after primary PCI

		Unadjusted		Adjusted*	
Genotype	Reference group	OR (95% CI)	*p* value	OR (95% CI)	*p* value
–786T>C polymorphism					
C/C	T/T	3.90 (0.87–17.45)	0.07	3.54 (0.49–25.21)	0.20
T/C	T/T	1.95 (0.64–5.91)	0.23	2.85 (0.64–12.59)	0.16
T/T	T/T	2.27 (0.79–6.50)	0.12	3.01 (0.73–12.32)	0.12
+894G>T polymorphism					
T/T	G/G	0.92 (0.21–3.98)	0.91		
T/G	G/G	0.62 (0.21–1.82)	0.39		
G/G	G/G	0.69 (0.26–1.84)	0.46		

eNOS: Endothelial nitric oxide synthase; PCI: Percutaneous coronary intervention; OR: Odds ratio; CI: Confidence interval.

Table 6. Adjusted and unadjusted effects of potential predictors of the coronary slow-flow phenomenon after primary PCI

	Unadjusted		Adjusted*	
Risk factor	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Platelet count	1.00 (0.99–1.00)	0.07	1.00 (0.99–1.00)	0.06
Blood sugar on admission	1.00 (1.00–1.01)	0.02	1.00 (0.99–1.01)	0.11
Hemoglobin level	0.70 (0.54–0.99)	0.04	0.71 (0.48–1.05)	0.08

PCI: Percutaneous coronary intervention; OR: Odds ratio; CI: Confidence interval.

evaluated the role of genetic predisposing factors in the occurrence of CSFP,[29,35–37] there are limited data on their role in a setting of STEMI.

The no-reflow phenomenon is a feared complication after PPCI; it is associated with a poor long-term prognosis and is regarded as an independent predictor of death, MI, and impaired left ventricular function.[38] Various treatments have been tested for the no-reflow phenomenon; however, the complex and multifactorial pathogenesis of this phenomenon limits the efficacy of these therapies.[14,39] Hence, it is essential that patients at higher risk of the no-reflow phenomenon be identified and treated early. In this regard, several studies have demonstrated that biomarkers and other clinical parameters could be helpful in the risk assessment and identification of high-risk patients.[11]

Endothelial dysfunction may play a key role in the pathophysiology of the no-reflow phenomenon. NO is synthesized by eNOS and has a regulatory function in vasomotor tone and blood flow.[4] Endothelial NO has the ability to inhibit platelet activation and leukocyte adhesion and to modulate the growth of the vascular smooth muscle.[4,40]

Previous studies have indicated that NOS3 polymorphisms can affect both the production and the function of NO and may cause endothelial dysfunction.[29,41,42] Therefore, polymorphisms of the eNOS gene are considered to be a risk factor for this phenomenon.[43] Hingorani et al.[40] were the first investigators to observe that a point G/T mutation in exon 7 of the NOS3 polymorphism (894G>T) was correlated with CAD and recent MI. Ensuing investigations revealed that the mentioned SNP might be related to the occurrence of MI,[44] the increased risk of coronary spasm,[25] and the incidence of essential hypertension.[45] Subsequent studies reported mixed results about the role of the 894G>T polymorphism in CAD inasmuch as some investigations allied the SNP to a lower intracellular NO production rate,[45,46] whereas others rejected any relationship.[47] The same situation exists for the other common NOS gene variant, -786T>C. In their early report, Nakayama et al.[48] demonstrated that the SNP

reduced NOS promoter activity by 50% and was associated with coronary spasm. Similarly, conflicting results have been published on the role of -786T>C: While some investigators have reported a strong relationship to the occurrence and severity of CAD,(10) others have arrived at no such conclusion.[40,47–49] Rai et al.,[43] in their systematic review of the association between polymorphisms of the eNOS gene and CAD, showed that ethnic variety influenced the effects of different SNPs on the risk of CAD. They found that 894G>T had the strongest relationship in their Middle Eastern subgroup and -786T>C showed the highest association with CAD among their population with Asian ancestry. As stated previously, in our study, the small number of patients (n=4) complicated by the no-reflow phenomenon (TIMI flow grade=0-1) rendered a statistical analysis in this regard meaningless and forced us to compare the genetic variety of 2 of the most common eNOS SNPs (i.e., 894G>T and -786T>C) between patients with normal post-PPCI TIMI flow (≥3) and those with CSFP (post-PPCI TIMI flow ≤2). Our analysis indicated that the 894G>T polymorphism was not significantly associated with post-PPCI CSFP. Regarding the -786T>C polymorphism, our patients with the C/C genotype had a marginally significant risk of CSFP. This effect was not consistent in our adjusted analysis. To our knowledge, the existing literature contains no investigation of the role of the polymorphisms of the eNOS gene in a PPCI setting. The majority of previous works have focused on the relationship in patients with stable CAD in whom CSFP was detected in coronary angiography. Even in this circumstance, however, mixed results have been reported on the association between CSFP and polymorphisms of the eNOS gene. Gazi et al.[35] found that endothelial function was impaired in their patients with CSFP, but eNOS gene polymorphism (-786T>C) was not associated with CSFP. Caglayan et al.[50] reported the absence of an association between the 894G>T polymorphism and T-allele frequency of the eNOS gene and the presence of CSFP in a Turkish population. In contrast, Nurkalem et al.[36] reported an association between CSFP and the -786T>C polymorphism of the eNOS gene, in addition to a positive correlation between the TIMI frame count and the C allele. Ekmekci et al.[29] posited that the presence of the allele 'a' in intron 4a/b polymorphism of the eNOS gene might be a risk factor for microvascular endothelial dysfunction in patients with CSFP and reported that the allele 'a' was correlated with a higher TIMI frame count.[29] Gupta et al.[37] reported a significant association between the 894G>T polymorphism and CSFP and reported a trend toward lower NO levels as the frequency of the T allele increased.

Several reasons can be cited for the lack of association found in our study. For instance, 894G>T is not localized within the functional domain in the eNOS gene sequence,[45] which might cause the poor effect. Moreover, a meta-analysis by Rai et al.[43] showed that this diverse association between polymorphisms of the eNOS gene might be related to the variety of populations and gene pools. Interestingly, 2 other Iranian studies did not find any association between NOS3 polymorphisms and CAD.[51,52]

Apart from the association between the no-reflow phenomenon and CSFP and polymorphisms of the eNOS gene, several studies have endeavored to define the role of demographic and clinical characteristics in conjunction with procedural factors as independent predictors of this phenomenon after PPCI. Among the demographic characteristics, age,[14] female gender,[53] and cigarette smoking[43] were the most common risk factors allied to no-reflow/ CSFP. In our investigation, we found a lower prevalence rate of cigarette smoking in our patients with post-PCI CSFP. Chest pain duration has been proposed as an important predictor of the no-reflow phenomenon in multiple studies, which have demonstrated that a delayed presentation and an increased reperfusion time have a hazard ratio of 1.72 (95% CI: 1.21–2.24; p=0.0024) and an OR of 13.84 (95% CI: 3.21–59.63; p<0.001).(14) We, however, observed no significant effect regarding chest pain duration on the incidence of CSFP following PPCI. Ipek et al.[9] were the first to evaluate the power of the CHA2DS2-VASc score in predicting no-reflow/ CSFP. In our study, the CHA2DS2-VASc score was not a relevant estimator to predict CSFP in patients following PPCI. Several laboratory findings have been suggested as potential predictors of the no-reflow phenomenon in patients undergoing PPCI. Iwakura et al.[12] reported that the no-reflow phenomenon was more frequent in patients with a blood glucose level ≥160 mg/dL than in those without hyperglycemia.. Intriguingly, however, they found no difference in the incidence of diabetes mellitus between the 2 subsets. Likewise, we observed that our patients with CSFP after PPCI had significantly higher blood sugar levels on admission. Adding to other reports, our results

revealed no difference pertaining to the incidence of diabetes mellitus between our CSFP and NF groups.

Of the angiographic indices, the thrombus grade and the SYNTAX score have been assessed the most. Mazhar et al.[14] reported a hazard ratio of 2.28 (95% CI: 1.50–3.55; p<0.0001) for the thrombus grade. We found no such relationship in the present study. Magro et al.[15] were the first investigators to study the role of the SYNTAX score in predicting the no-reflow phenomenon (OR: 1.29; 95% CI: 1.02–1.63; p<0.001). However, we could not observe a predictive effect of the SYNTAX score on the incidence of CSFP following PPCI. This may be due to the smaller number of subjects with intermediate and high SYNTAX scores in the present study.

In conclusion, our study showed no relationship between post-PPCI CSFP and Glu298→Asp (894G>T) and -786T>C, which constitute 2 of the most common polymorphisms of the eNOS gene. By contrast, some clinical predictors, such as the blood sugar level on admission, the platelet count, and the hemoglobin level, had a better risk discriminatory influence. These potential clinical predictors are easier and less expensive to measure than more sophisticated genetic analyses. However, the true value of these predictors (clinical and genetic), should be tested in large scale studies for final discrimination.

Limitations

The major limitations of this research are its limited sample size and observational nature. Given the low incidence of the no-reflow phenomenon, several studies have expanded the definition of no-reflow and performed their final analysis on patients with CSFP (TIMI flow grade <3).[8,54,55] In the present study, we adopted this expanded definition. Interestingly, pivotal review articles have described the 2 categories as a spectrum of diseases that share the same physiopathology (e.g., distal embolization and microvascular dysfunction).[56–58] Although the classic no-reflow phenomenon is certainly a more devastating event with a poorer outcome and, thus, necessitates immediate treatment,[59] slow flow during PPCI has also been shown to influence the prognosis.[55] A stronger association might emerge in future studies with larger sample sizes of patients with the classic no-reflow phenomenon. The results of the present study should, therefore, be confirmed by larger studies.

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Keywords: Endothelial nitric oxide; gene polymorphism; no reflow; primary percutaneous coronary intervention; slow flow; ST-segment elevation myocardial infarction.

Anahtar sözcükler: Endotelyal nitrik oksit; gen polimorfizmi; akımsızlık; primer perkütan koroner girişim; yavaş akım; ST segment elevasyonlu miyokart enfarktüsü.