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Research Article/Araştırma Makalesi

The effect of smoking on platelet reactivity assessed by p2y12 platelet function testing in patient with clopidogrel therapy

Sigara kulanımının klopidogrel tedavisi alan hastalarda p2y12 trombosit fonksiyon testi ile değerlendirilen trombosit reaktivitesi üzerine etkisi

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ABSTRACT

Aim: The interaction between cigarette smoking and efficacy of platelet function testing is not definitely shown. We aimed to investigate the effects of smoking on platelet reactivity by P2Y12 platelet function testing on the patients treated with clopidogrel.

Material and Method: This prospective study contained 200 patients who underwent a previous stent implantation. Diagnostic coronary angiography was performed those patients due to chest pain. The clopidogrel resistance test was applied. Furthermore, the smoking effect on platelet reactivity was investigated. Then stent restenosis rates were investigated.

Results: We found that 17% of the patients (34/200) clopidogrel-resistant by P2Y12 platelet function test. We didn't find any relationship between angiographic stent restenosis and clopidogrel resistance ($p>0.05$). There was statistically significant relationship between smoking and clopidogrel resistance. The smokers had also higher platelet reactivity level (>208 PRUs) as revealed by VerifyNow P2Y12 assay.

Discussion: The test results achieved through the clopidogrel resistance by P2Y12 platelet function testing did not meet expectation with the angiographically observations.

Conclusion: P2Y12 platelet function testing can be unclear in smoker patients.

Keywords: Clopidogrel resistance, coronary artery disease, smoking

ÖZ

Amaç: Sigara içimi ve trombosit fonksiyon testinin etkinliği arasındaki etkileşim kesin olarak gösterilmemiştir. Bu çalışmada klopidogrel ile tedavi edilen hastalarda sigara içiminin P2Y12 trombosit fonksiyon testi ile belirlenen trombosit reaktivitesi üzerine etkilerini araştırmayı amaçladık.

Gereç ve Yöntem: Bu prospektif çalışmaya daha önce stent implantasyonu yapılan ve klopidogrel kullanan 200 hasta dahil edildi. Bu hastalara göğüs ağrısı nedeniyle tanısal koroner anjiyografi yapıldı ve klopidogrel rezistans testi uygulandı. Ayrıca, trombosit reaktivitesi üzerindeki sigara etkisi araştırıldı.

Bulgular: P2Y12 trombosit fonksiyon testi ile hastaların %17'sinin (34/200) klopidogrel dirençli olduğunu bulduk. Anjiyografik stent restenoz ile klopidogrel direnci arasında ilişki tespit etmedik ($p>0.05$). Ancak sigara içme ve klopidogrel direnci arasında istatistiksel olarak anlamlı ilişki vardı. Yine sigara içenlerde VerifyNow P2Y12 testiyle tespit edilen trombosit reaktivite seviyeleri de yüksekti (>208 PRU).

Tartışma: Sigara içenlerde P2Y12 trombosit fonksiyon testi ile tespit ettiğimiz klopidogrel direnci beklendiği gibi stent restenoz oranlarında yükseklik ile paralellik göstermedi.

Sonuç: Sigara içenlerde P2Y12 trombosit fonksiyon testi klinik bulgularla uyumlu olan net sonuçlar vermeyebilir.

Anahtar Kelimeler: Klopidogrel direnci, koroner arter hastalığı, sigara kullanımı

INTRODUCTION

Although positive developments in coronary stent technology for percutaneous coronary intervention have occurred since 1986, there remain certain unresolved conditions (1). One of them is in-stent restenosis (ISR). Basic risk factors for ISR refer to patient-related (smoking e.g), stent type-related, and procedure-related risk factors. One of the causes of the patient-related ISR is insufficient P2Y12 inhibition. Having been used since 2000s in order to prevent stent restenosis, clopidogrel inhibits platelet aggregation by blocking platelet adenosine diphosphate P2Y12 receptor (2). However, inadequate platelet inhibition by clopidogrel may cause an increase in cardiovascular events (3). To solve this problem, using newer P2Y12 receptor antagonist in acute coronary syndrome and acute myocardial infarction (4,5), or use after clopidogrel resistance testing can be thought.

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Another risk factors, cigarette smoking is still a problem for cardiovascular morbidity and mortality. The data on the association of smoking and platelet responsiveness to adenosine diphosphate receptor blocking drugs like clopidogrel are inconsistent (6). Thus, we aimed to examine effects of smoking on platelet reactivity by P2Y12 platelet function testing on the patients treated with clopidogrel.

In the present study, we aimed to investigate the effects of smoking on platelet reactivity by P2Y12 platelet function testing on the patients treated with clopidogrel.

MATERİAL AND METHOD

Ethics Statement

This prospective study was approved by Medicana International Ankara Hospital the Ethics Committee [Number, 2017/1] and and reporting of the study conforms to STROBE statement along with references to STROBE statement and the broader EQUATOR guidelines. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Study Population

We enrolled 200 patients who had symptom and findings for stable ischemic heart disease according to 2014 ACC/AHA update of the guideline for stable ischemic heart disease (7) and coronary stent history between May 2017 and March 2018. we performed diagnostic coronary angiography those 200 patients. The patients were studied in terms of clopidogrel resistance and stent restenosis. The patient with acute coronary syndrome history and congestive heart failure were excluded.

Clopidogrel Resistance Test

Clopidogrel inhibition level was measured from venous blood samples collected 12-24 hours after the procedure. The inhibitory effect of clopidogrel was measured by VerifyNow P2Y12 assay, which primarily measures the effect of the drug on the P2Y12 receptor. In testing, P2Y12 reaction units (PRUs) were used. The patients with >208 PRU were considered resistant (8), while those with lower levels were considered responsive to clopidogrel.

***Definition of in-Stent Restenosis (ISR)*:** It refers to a condition during which a 50 % narrowing in stent diameter occurs angiographically.

***Smoking Definition*:** Cigarette smoking habit was defined as regular smoking 10 cigarettes a day for at least 3 months.

Patients were included in the study receiving clopidogrel and aspirin following stent implantation. Mean clopidogrel used time had 16.34±2.55 months. All patients intake 75 mg/daily clopidogrel and 100 mg aspirin, after loading 600 mg clopidogrel dosage according to previous medical report.

Patients were divided into clopidorel resistant and clopidogrel responsive groups according to P2Y12 platelet function testing. Then effect of smoking was evaluated and stent restenosis rates were investigated.

Statistical Analysis

Statistical analyses were performed using SPSS software (version17.0; SPSS Inc., Chicago, IL, USA). Continuous variables were presented as mean ±SD. Student's t-test was used to compare mean variables among groups for parametric assumptions. Intergroup comparisons of categorical data were performed using continuity-corrected chi-square or Fisher's exact test. A p value of <0.05 was considered statistically significant.

RESULTS

Two hundred consecutive patients were included in the study. Totally 243 stent implantations were applied these 200 patients. The baseline characteristics of the study population are shown in **Table 1**.

Table 1. The clinic and angiographic features of patients

Variable	Value	Frequencies (n)	Percent (%)
Gender	Female	53	26.5
	Male	147	73.5
Diabetes mellitus	No	160	80.0
	Yes	40	20.0
Hypertension	No	107	53.5
	Yes	93	46.5
Smoking	No	148	74.0
	Yes	52	26.0
Before stent availability	RCA	67	33.5
	Cx	56	28.0
	LAD	69	34.5
	Two vessel disease	3	1.5
	Saphenous vein graft stent	5	2.5
Stent thrombosis	Yes	9	4.5
	No	191	95.5
Clopidogrel resistance	Yes	34	17.0
	No	166	83.0
Stent type	Bare	25	12.6
	Drug-eluting	174	87.4
Stent restenosis	Yes	9	4.5
	No	191	95.5

Abbreviations: RCA: Right coronary artery, Cx: Circumflex coronary artery, LAD: Left anterior descending coronary artery.

Male gender was found 73.5% of the patients The presence of diabetes mellitus was 20% and presence of hypertension was 46.5% of the patients. Smokers ratio was 26% in the sudy population. The patients had second generation drug eluting stent (174, 87.4%) or bare metal stent (25, 12.6%). Stent length was 21.13±4.86 mm (min 11-max 32 mm) and reference vessel diameter was 2.83±1.86 mm (min 2.25-max 3.5 mm). Clopidogrel resistance was found

Table 2. Comparison of clinical features between clopidogrel-responsive and resistant groups

n=200	Clopidogrel resistance						*P*
	Responsive (n=166)			Resistant (n=34)			
	n	%	Mean±SD	n	%	Mean±SD	
Agea			61.35±10.23			58.29±11.98	0.125
Male	118	80.3		29	85.2		0.087
Diabetes mellitus	0.087	21		5	15		0.397
Hypertensionb	78	46		15	44		0.760
Smokingb	31	18		21	61		0<0001
Stent typec							
Bare metal stent	21	12.0		4	11.0		0.570
Drug-eluting stent	144	86.0		30	88.0		0.510
Stent restenosisc	7	4.2		2	5.8		0.472

Abbreviations: SD: Standard deviation; +: Indicates presence;-: Indicates absence.
a Indicates performance of Student's t-test; b Indicates performance of continuity-corrected chi-square test; c Indicates performance of Fisher's exact test.

in 34 (17%) of patients. No statistically significant difference was found between groups regarding age, diabetes mellitus, and hypertension between resistant and responsive groups (p>0.05). Stent restenosis rate had 4.5%. and both group had similar results in terms of stent restenosis (p>0.05).

While the clopidogrel-responsive and the clopidogrel-resistant groups were compared (**Table 2**) there was no significantly differences in terms of age, gender, diabetes mellitus, hypertention, stent type and stent restenosis.

The number of smokers in study population was 52 (26%). The number of smokers patients with clopidogrel responsive were 31 (16%) and the number of smoker patients with clopidogrel resistant were 21 (11%) of study population. While the ratio of smokers in clopidogrel-responsive group was 18% and in clopidogrel resistance group was 62% (p < 0.0001). Although the smokers had significantly higher P2Y12 Reaction Units than nonsmokers (p < 0.05) (**Figure 1**).

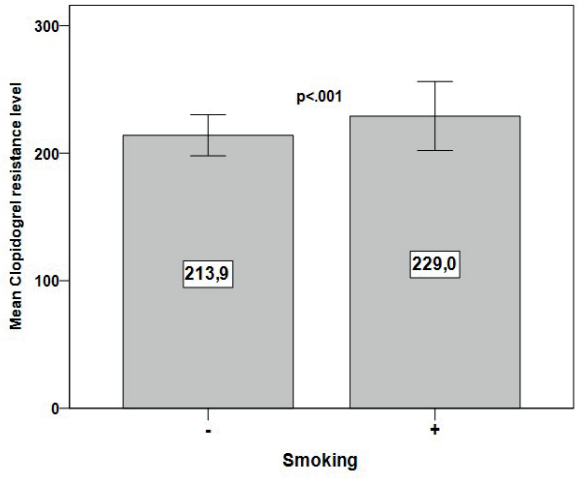

Figure 1. Mean clopidogrel resistance levels of smoking and non-smoking patients.

In **Table 3**, the smoking effect was examined. Clopidogrel resistance was singificantly higher in the smoker patients (p < 0.0001) but tehere was no signicantly diference in stent restenosis between two groups (p = 0.196).

Table 3. Smoking effects on clopidogrel resistance and stent restenosis.

n=200	Clopidogrel resistance				
	Responsive (n=166)		resistant (n=34)		
Smoking	n	%	n	%	*P*
-	135	82	13	38	<0,0001
+	31	18	21	62	
	Stent restenosis				
	Yes (n=10)		No (n=190)		
Smoking	n	%	n	%	*P*
-	5	3.6	132	96.4	0.196
+	5	7.9	58	92.1	

DISCUSSION

The ISR predictors are small vessels, residual stenosis after stenting, longer stented length. Especially, the presence of diabetes mellitus is usually considered as an important ISR and clopidogrel resistant factor. Stent type-related factors can also play a more important role (9). DESs are very effective in reducing the chances of restenosis. However, the process of stent endothelialization also gets delayed by several months or years (10), and the stents have been found to be incompetently covered with neointima with thrombi over bare metal surface of the stent (11-13).

Recently, growing awareness of response to ADP-antagonist therapy has concentrated on predicting this risk. Therefore, both pharmacogenetic and platelet function

testing are attractive for identifying patients at risk. Clopidogrel resistance has been characterized by variation in intestinal absorption, alteration in metabolism via hepatic CYP450 enzyme, variation in the combination with its platelet P2Y12 receptor, and possible interaction with other drugs. The prevalence of clopidogrel resistance in various studies was 5%-44% (14).

ISR risk prediction was investigated in terms of the association between inflammatory biomarkers at the time of stenting. The inflammatory response to coronary stenting as assessed by the variation in CRP was found correlation with the development of in-stent restenosis (15). While clopidogrel resistance was proven to cause poor results (e.g., stent thrombosis), its effect on SR is not known.

The data on the association of smoking and platelet responsiveness to adenosine diphosphate receptor blocking drugs like clopidogrel are inconsistent and controversial with studies variably showing: 1) no association; 2) a positive (higher on-treatment platelet reactivity in smokers) association; or 3) a negative (lower on-treatment platelet reactivity in smokers) association (6). Cigarette smoking is an inducer of cytochrome P450 1A2, a hepatic enzyme involved in clopidogrel metabolism. Ueno et al. found that cigarette smoking is associated with a dose-response effect on clopidogrel-induced antiplatelet effects and lower rates of platelet reactivity in diabetes mellitus patients (16). Gremmel at al. (17) investigated the influence of cigarette smoking on clopidogrel-and aspirin-mediated platelet inhibition after percutaneous intervention with stent implantation. They claimed by in vitro testing, cigarette smoking is associated with enhanced clopidogrel-mediated platelet inhibition. Kim et al. (18) did extensive analyses to examine 1,314 patients undergoing PCI (pooled from 3 cohorts) by VerifyNow P2Y12 Assay and found none association.

However, the recently published COPTER (Cigarette Smoking on Platelet Reactivity) study by Patti et al. (19) assessed the effects of smoking on platelet reactivity among smokers with recent percutaneous coronary intervention for ST-segment elevation myocardial infarction and chronic treatment with clopidogrel. Patti et al. (19) found a significant reduction of PRU by VerifyNow P2Y12 assay values after a 15-day period of smoking cessation and increase of platelet reactivity after a further 15 days of smoking resumption. In our study, smoker patients had also higher platelet reactivity level (>208 PRUs) by VerifyNow P2Y12 assay. We thought that the clopidogrel resistance was masked by smoking induced cytochrome P450 and its associated clopidogrel metabolism. The cut-off value of PRU in this study was 208. PRU value has some important topics. Firstly, it is the highest in the acute phase after PCI and decreases after in ACS patients (after 3 days). Secondly, because CYP2C19 polymorphisms are more frequent in Asian populations than in Western populations (20), PRU value is higher in Asian populations.

CONCLUSION

The laboratory results obtained through the clopidogrel resistance by P2Y12 platelet function testing do not match with the angiographically observations. Moreover, smoker patients had higher platelet reactivity level. Therefore, P2Y12 platelet function testing prediction can be confusing in smoker patients. However, further large-scale studies are required to generalize these findings.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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