

ORIGINAL ARTICLE

Association between high on-treatment platelet reactivity to clopidogrel and hepatosteatosi in patients undergoing elective stent implantation

Elektif olarak stent yerleřtirilen hastalarda klopidogrel tedavisi sırasında yüksek trombosit reaktivitesi ile karacięer yaęlanması arasında iliřki

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ABSTRACT

Objective: The present study is an investigation of the association between high on-treatment platelet reactivity to clopidogrel (HTPRC) and hepatosteatosi in patients who had elective stent implantation due to coronary artery disease.

Methods: A total of 190 consecutive patients who underwent an elective coronary stent implantation due to coronary artery disease were prospectively enrolled in the study. Eligible patients were given a 300 mg loading dose of clopidogrel before percutaneous coronary intervention. All of the patients underwent an ultrasound assessment for fatty liver. The patients were divided into 2 groups according to the detection of HTPRC: patients with HTPRC and patients without HTPRC.

Results: HTPRC was present in 54.2% (103 of 190 patients) of the total study population. The age and body mass index data were similar between the 2 groups. In all, 111 (58.6%) patients had hepatosteatosi. The HTPRC ratio was statistically higher in female patients ($p=0.032$). Hepatosteatosi was significantly greater in patients with HTPRC ($p<0.001$); 84 (81.6%) patients with HTPRC had hepatosteatosi ($p=0.001$). There was also a statistically significant association between the hepatosteatosi grade and HTPRC ($p<0.001$). The percentage of HTPRC was greater in patients with \geq grade 2 hepatosteatosi than grade 1 ($p<0.001$). Logistic regression analysis indicated that hepatosteatosi (odds ratio: 9.403, 95% confidence interval: 4.519–19.566; $p<0.001$), fasting blood glucose, and hypertension were independent predictors of HTPRC.

Conclusion: To the best of our knowledge, this is the first study to demonstrate a relationship between hepatosteatosi and HTPRC.

ÖZET

Amaç: Çalışmamızda koroner arter hastalığı (KAH) nedeniyle elektif olarak stent yerleřtirilen hastalarda klopidogrel tedavisi sırasında saptanan yüksek trombosit reaktivitesi ile karacięer yaęlanması arasındaki iliřkinin araştırılması amaçlandı.

Yöntemler: Koroner arter hastalığı nedeniyle elektif olarak koroner stent yerleřtirilen ardışık 190 hasta ileriye yönelik olarak çalışmamıza alındı. Uygun hastalara perkütan koroner girişim (PKG) öncesi 300 mg klopidogrel yükleme dozu verildi. Tüm hastalara karacięer yaęlanmasının tespiti için ultrasonografi yapıldı. Hastalar klopidogrel tedavisi sırasında yüksek trombosit reaktivitesinin saptanıp saptanmamasına göre iki gruba ayrıldı: Dirençli hastalar ve dirençsiz hastalar.

Bulgular: Klopidogrel tedavisi sırasında yüksek trombosit reaktivitesi (HTPRC) tüm çalışma hastalarının %54.2'sinde (190 hastanın 103'ünde) görüldü. Yaş ve vücut kitle indeksi verileri iki grup arasında benzerdi. Karacięer yaęlanması 111 (%58.6) hastada saptandı. HTPRC oranı kadın hastalarda istatistiksel olarak daha yüksekti ($p=0.032$). HTPRC olan hastalarda karacięer yaęlanması anlamlı derecede daha fazlaydı ($p<0.001$). HTPRC olan 84 (%81.6) hastada hepatosteatoz mevcuttu ($p<0.001$). Bunun yanında, karacięer yaęlanması derecesi ve HTPRC arasında istatistiksel olarak anlamlı pozitif korelasyon mevcuttu ($p<0.001$). HTPRC yüzdesi \geq Evre 2 karacięer yaęlanması olan hastalarda Evre 1 karacięer yaęlanması olanlara göre daha yüksekti ($p<0.001$). Lojistik regresyon analizinde karacięer yaęlanması [odds oranı (OR) 9.403, %95 güven aralığı 4.519–19.566, $p<0.001$], açlık kan şekeri ve hipertansiyon HTPRC'nin bağımsız öngördürücüleri olarak belirlendi.

Sonuç: Bu çalışma karacięer yaęlanması ve HTPRC arasındaki iliřkiyi gösteren ilk çalışmadır.

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The cornerstone of treatment for patients presenting with acute coronary syndrome and for those undergoing percutaneous coronary intervention (PCI) consists of dual anti-platelet therapy with aspirin and an antagonist of the P2Y12 receptor.

^[1] Although new antiplatelet agents have recently emerged, clopidogrel is still the most widely used P2Y12 receptor antagonist. However, high on-treatment clopidogrel platelet reactivity (HTPRC) is a common phenomenon that is associated with adverse clinical consequences, such as increased stent thrombosis risk.^[2,3]

Polymorphisms in the CYP2C19 gene have been shown to account for around 12% of the between-subject variability to clopidogrel treatment response. The variability has been attributed to age and body mass index (BMI) in 3.8% and 2.3% of patients, respectively.^[4] Other studies have demonstrated similar findings, indicating the contribution of multiple demographic and risk factors to the inter-individual variability in response to clopidogrel treatment.^[5-7] The underlying mechanisms of these intrinsic and extrinsic factors are not fully known.

Several cellular abnormalities can lead to increased basal platelet activation. These include inflammation and resultant pro-thrombotic adipokine release, impaired absorption of clopidogrel, reduced production of active metabolites, and drug under dosage, all causing a reduced response to the drug.^[8,9] Both diabetes mellitus (DM)^[10] and increased BMI^[11] have been associated with HTPRC. Obesity has a strong association with metabolic syndrome (MS) and DM, and HTPRC may be a common phenomenon over the spectrum of insulin resistance.

Hepatosteatosis has a strong association with insulin resistance (IR) and plays a role in the pathogenesis of type 2 DM and MS.^[12] Patients with hepatosteatosis have been found to be resistant to insulin at the liver and muscle level in previous studies.^[13,14] Hepatosteatosis, which is a common phenomenon, affects up to 30% of adults and up to 10% of children in developed countries.^[15] This study was an exami-

nation of the association between HTPRC and hepatosteatosis in patients with coronary artery disease.

METHODS

Study design and patient selection

In all, 205 patients in whom critical coronary artery stenosis was detected during elective coronary angiography performed between January 2014 and December 2014 and who then had elective stent (bare metal stent or drug-eluting stent) implantation were prospectively and consecutively evaluated. None of the patients included in the study had acute coronary syndrome. Coronary angiography was performed in order to search for possible coronary artery disease (stable coronary artery disease), suspected as a result of noninvasive tests (electrocardiography, exercise test, transthoracic echocardiography, scintigraphy, etc.) done to evaluate symptoms such as chest pain and shortness of breath. Patients with a finding of critical coronary artery stenosis during coronary angiography who subsequently underwent elective stent implantation were given a 300 mg loading dose of clopidogrel and 300 mg acetyl salicylic acid (ASA) orally on the day of the procedure. All patient blood samples to be tested for clopidogrel efficiency were collected in 4.0 mL plastic tubes containing lepirudin (25 mg/mL, Refludan, hirudin blood collection tubes; Dynabyte Medical, Munich, Germany). The literature does not provide definitive data regarding the most appropriate time for blood to be drawn to test for platelet reactivity after clopidogrel dose. However, in the majority of studies, blood was drawn in the first 24 hours after the administration of a loading dose of clopidogrel.^[16,17] Blood samples were collected between 12 and 24 hours after the clopidogrel loading dose was provided to our study population. The loading dose was followed by a maintenance dose of 75 mg clopidogrel daily and 100 mg ASA daily. An elective PCI was performed 72 to 96 hours after the coronary angiography was performed as an institutional protocol. The femoral route was used for all PCI procedures. Unfractionated heparin (70 IU/kg) was administered at the beginning of the intervention, and if the procedure was prolonged, an additional dose of heparin was administered to ensure an activated clotting time of 250 to 300 seconds. If needed, pre-dilation using balloon angioplasty was performed before the stent deployment. A drug-eluting stent or a bare metal stent was implanted depending on the lesion

Abbreviations:

ADP	Adenosine diphosphate
ASA	Acetyl salicylic acid
BMI	Body mass index
CI	Confidence interval
DM	Diabetes mellitus
HTPRC	High on-treatment platelet reactivity to clopidogrel
IR	Insulin resistance
MS	Metabolic syndrome
PCI	Percutaneous coronary intervention
TAG	Triacylglycerol

complexity, co-morbidities of patient, patient preference, and the surgeon's preference. The samples were studied with a Multiplate platelet function analyzer (Dynabyte Medical, Munich, Germany). Electrical impedance of multiple electrodes was used to measure platelet aggregation. An adenosine diphosphate (ADP) test (20 μ L test reagent) and an arachidonic acid test (20 μ L test reagent) were employed to assess platelet response to clopidogrel using a Multiplate aggregometry analyzer (whole blood impedance platelet aggregometer, Dynabyte Medical, Munich, Germany). The overall basal capacity of platelet aggregation was assessed with a thrombin receptor activating peptide test (20 μ L test reagent). Platelets, which are non-thrombogenic in the resting phase under normal circumstances, expose their surface receptors when activated by 20 mmol/L of ADP. This enables the platelets to adhere to vessel walls and artificial surfaces. Platelets bind to sensor wires in the ADP-supplemented Multiplate test tubes, resulting in an increase in the resistance of the sensor. This increase in the resistance is interpreted as the computed aggregation unit in a unit versus time graph. The results were obtained using the Multiplate analyzer and were expressed as the area under the aggregation curve. Dynabyte Medical supplied all of the materials utilized for this purpose. HTPRC was defined with a cut-off value of >470 area under the aggregation curve per minute for clopidogrel, as described by the manufacturer. The patients were divided into 2 groups according to the presence of HTPRC: those with HTPRC and those without.

Patients under 18 years of age, patients with acute coronary syndrome, those already on aspirin or clopidogrel treatment, patients with active major or minor bleeding after the loading dose of clopidogrel or ASA, those with hemodynamic instability, a cerebrovascular accident in the previous 3 months, active malignancy, autoimmune disease, hematological proliferative disease, known immune deficiency, pregnancy or lactation, those who had used nonsteroidal anti-inflammatory drugs within the previous 7 days, those with a history of recent bleeding or a bleeding diathesis, or a proven allergy to clopidogrel or contrast media were excluded from the study. Exclusion criteria also included patients with an active or chronic inflammatory disease, those with a platelet count $<100 \times 10^6 \mu$ L/mL, a hemoglobin level <10 g/dL, a creatinine level >2.5 mg/dL, those with liver disease (bilirubin level >2 mg/dL), patients who had used parenteral or oral

anticoagulation in the 30 days prior, and those who had used cytochrome P-450-metabolized drugs (fluconazole, ketoconazole, voriconazole, etravirine, felbamate, fluoxetine, fluvoxamine, omeprazole, esomeprazole, lansoprazole, rabeprazole). The exclusion criteria eliminated 15 patients; the data of 190 patients were included in the analysis. The demographic and clinical features of all patients were recorded. Compliance with prescribed drugs, including aspirin and clopidogrel, was assessed using a questionnaire at a follow-up visit. Routine blood biochemistry, hematological parameters, thyroid function tests, and enzyme-linked immunosorbent assay tests for HIV, hepatitis B virus, and hepatitis C virus were performed before the coronary angiography. All of the patients had undergone an ultrasound examination to detect the presence of fatty liver by the same specialist.

Definitions

The definition of DM was a previous diagnosis, anti-diabetic drug use at hospital admission, or the presence of at least 2 fasting blood sugar measurements of >126 mg/dL during hospitalization. A previous diagnosis of hypertension or a history of antihypertensive drug use, or a systolic pressure measurement of ≥ 140 mm Hg or a diastolic pressure reading of ≥ 90 mm Hg on at least 2 separate occasions while hospitalized but at least 48 hours after the index event were used to define hypertension. Weight and height were measured, and BMI was calculated as follows: $BMI = \text{weight} / \text{height}^2$ (kg/m²).

All of the patients had undergone a fatty liver test ultrasound examination. The ultrasound was performed by a single specialist using the same probe and 3.5-MHz scanner attached to a high-resolution ultrasound machine (Aplio; Toshiba Medical Systems Corp, Otawara, Japan). The ultrasonographic definition of steatosis was the appearance of hyperechoic liver parenchyma with tightly packed fine echoes and posterior beam attenuation. The severity of steatosis was graded as mild (grade 1), moderate (grade 2), or severe (grade 3) using these criteria.^[18-20] A liver with normal echotexture and clearly defined, visualized internal vascular structures was classified as normal. A slight increase in parenchymal echogenicity and a slightly decreased definition of the portal vein walls with minimal or no posterior beam attenuation was classified as grade 1 steatosis. A grade of 3 was assigned when grossly increased hepatic parenchy-

mal echotexture was observed, visualization of only the main portal vein walls (absence of all smaller venules), and marked posterior beam attenuation. Grade 2 steatosis was used to describe features between those considered mild and severe. This study was conducted in accordance with the principles of the Declaration of Helsinki and with the approval of the institutional ethics committee. All of the patients provided written informed consent.

Statistical analysis

All of the study data were evaluated using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA). The pattern of distribution for continuous variables was determined with a one-way Kolmogorov-Smirnov test. An independent samples t-test (Student's t-test) was used for group comparison of normally distributed continuous variables, and if the distribution was not normal, the Mann-Whitney U test was used. The Pearson chi-square test and the Yates correction for continuity were used for categorical variables. Continuous variables were presented as the mean \pm SD if the independent samples test was used, and median (min-max) if the Mann-Whitney U test was used. Categorical data were expressed as a percentage of the total. Candidate predictors for HTPRC were identified based on the opinion of Hosmer and Lemeshow stating that variables with a p value of <0.25 following univariate analysis may be statistically significant in multiple variable logistic regression analysis. Thus, these predictors were included in the multiple variable logistic regression model. The factors most predictive for HTPRC were identified using the backward logistic regression method. For all comparisons, a p value of <0.05 was accepted as significant. All of the data were within a 95% confidence interval (CI).

RESULTS

The study population consisted of 190 patients with a mean age of 55.58 ± 11.02 years. Of the total, 141 (74.1%) were male; 103 (54%) patients had HTPRC, and 111 (58.4%) patients had hepatosteato-

sis; however, only 27 (31%) patients without HTPRC had hepatosteato-

were more frequently female ($p=0.032$) (Table 1). There was a statistically higher level of fasting blood glucose, glycated hemoglobin, total cholesterol, low-density lipoprotein, and calcium, and a lower aspartate transaminase (AST) level in patients with HTPRC. Other biochemical parameters were similar between the groups (Table 1).

The presence of hepatosteato-

sized in patients with HTPRC ($p<0.001$). Eighty-four (81.6%) patients with HTPRC had hepatosteato-

sis; however, only 27 (31%) patients without HTPRC had hepatosteato-

sized (Table 1). There was a statistically significant association between hepatosteato-

sis grade and HTPRC, as shown in Table 1.

When candidate predictors for HTPRC of age, female gender, hypertension, fasting plasma glucose, total cholesterol, calcium, alanine transaminase, AST, hemoglobin, and hepatosteato-

sis were evaluated using the backward logistic regression method, the model was found to be significant and the explanatory coefficient was high (78.4%). The analysis indicated that hepatosteato-

sis [odds ratio (OR): 9.403; 95% CI: 4.519–19.566; $p<0.001$], fasting blood glucose (OR: 2.783; 95% CI: 1.249–6.201; $p=0.012$), and hyperten-

sion (OR: 2.962; 95% CI: 1.424–6.162; $p=0.004$) were independent predictors of HTPRC. AST remained in the model, but was not found to be statistically signifi-

cant ($p>0.05$) (Table 2).

DISCUSSION

The main findings of this study can be summarized as: i) this is the first report to evaluate the association between hepatosteato-

sis and HTPRC, ii) HTPRC was significantly higher in patients with hepatosteato-

sis, iii) there was a significant association between the grade of hepatosteato-

sis and the percentage of patients with HTPRC ($p<0.001$), and iv) hepatosteato-

sis was an independent predictor of HTPRC.

ADP, which plays a role in platelet activation, binds to platelets via G-protein receptors, namely P2Y1 and P2Y12. Stimulation of P2Y12 causes thromboxane A2 production, the secretion of thrombocyte alpha-granule ingredients, and the expression of P-selectin on the platelet cell wall.^[21] Clopidogrel is a selective and irreversible blocker of the P2Y12 receptor, which inhibits platelet activity and aggregation. Additionally, clopidogrel inhibits collagen and

Table 1. Baseline demographic details, laboratory measurements, and hepatosteatorosis grade of the 190 study subjects with respect to clopidogrel resistance

Parameters	Patients with HTPRC (n=103, 54%)	Patients without HTPRC (n=87, 46%)	<i>p</i>
Age, years	56.67±10.51	54.29±11.52	0.141 ^a
Body mass index (kg/m ²)	26.63±4.10	26.48±3.49	0.795 ^a
Female gender, n (%)	33 (32)	16 (18.4)	0.048 ^d
Diabetes mellitus, n (%)	32 (31)	4 (4.6)	<0.001 ^d
Hypertension, n (%)	70 (68)	34 (39.1)	<0.001 ^b
Hemoglobin (g/dL)	13.12±1.79	13.78±1.59	0.008 ^a
Fasting plasma glucose (mg/dL)	108 (78–374)	96 (68–292)	<0.001 ^c
Hemoglobin A1c (%)	6.11±1.54	5.60±0.57	0.004 ^a
Total cholesterol (mg/dL)	197.49±48.08	181.40±47.84	0.022 ^a
Triglycerides (mg/dL)	158 (38–690)	135 (59–765)	0.074 ^c
High-density lipoprotein (mg/dL)	39.01±12.18	38.19±9.53	0.612 ^a
Low-density lipoprotein (mg/dL)	122.12±38.13	110.75±39.97	0.047 ^a
Very low-density lipoprotein (mg/dL)	31 (7.6–138)	27 (11.8–153)	0.074 ^c
Calcium (mg/dL)	9.35±0.58	9.17±0.59	0.045 ^a
Sodium (mEq/L)	139.58±3.25	139.75± 2.93	0.716 ^a
Potassium (mEq/L)	4.41±0.47	4.38±0.47	0.661 ^a
Thyroid stimulating hormone (uIU/mL)	1.4 (0.25–4.7)	1.43 (0.02–4.82)	0.494 ^c
Aspartate aminotransferase (IU/L)	21 (9–280)	29 (14–849)	0.002 ^c
Alanine aminotransferase (IU/L)	23 (8–403)	28 (10–560)	0.082 ^c
Ultrasound findings			
Hepatosteatorosis (+), n (%)	84 (81.6)	27 (31)	<0.001 ^d
Hepatosteatorosis grade			<0.001 ^d
Normal, n (%)	19 (24.1)	60 (75.9)	
Grade 1, n (%)	55 (69.9)	24 (30.4)	
Grade 2 or more, n (%)	29 (90.6)	3 (9.4)	

^aIndependent samples t-test; ^bPearson chi-square test; ^cMann-Whitney U test; ^dContinuity (Yates) Corrected Chi-square test.
HTPRC: High on-treatment platelet reactivity to clopidogrel.

thrombin-induced aggregation.^[22] It is quickly absorbed from the intestine and converted to its active thiol metabolite by the cytochrome-P450 isoenzymes CYP3A4, CYP3A5, and 2C19.

The antiplatelet response to clopidogrel varies from patient to patient. HTPRC, though not yet precisely defined, is best determined by platelet activity before and after treatment, and is measured by the de-

Table 2. Factors predicting clopidogrel resistance in multiple variable logistic regression analysis

	Multivariate analysis		
	Odds ratio	95% Confidence interval	<i>p</i>
Hepatosteatorosis (+)	9.403	4.519–19.566	<0.001
Fasting plasma glucose (mg/dL)	2.783	1.249–6.201	0.012
Hypertension	2.962	1.424–6.162	0.004
Aspartate aminotransferase (IU/L)	0.995	0.989–1.001	0.128

gree of ADP-induced platelet aggregation *in vitro*.^[2] The variability of individual response to antiplatelet therapy remains a challenging clinical problem. There is still a significant number of patients who have adverse cardiovascular events after an elective coronary PCI.^[23] Multiple factors may contribute to these findings.^[24]

The prevalence of HTPRC varies from study to study and population to population; it has been estimated at between 5% and 56% of coronary stent implant patients in previous studies.^[25,26] These rates depend on the dosing, definition, laboratory methods, and the timing of blood sample collection.^[27] Gurbel et al.^[25] demonstrated the time-dependent feature of clopidogrel resistance. They reported that the incidence of HTPRC had a tendency to decrease over time: 53% to 63% of patients had clopidogrel resistance at the second hour, 30% on the 11th day, and 13% to 21% on the 30th day of therapy. In the present study, the prevalence of HTPRC was 54.2%. The patient blood samples were drawn between 12 and 24 hours after the clopidogrel loading dose in our study population, which could explain the higher HTPRC rate.

The etiology of clopidogrel response variability is multifactorial; genetic variations, such as CYP2C19 loss-of-function allele carriage, accounts for only 5% to 12% of the overall variability.^[4,28] CYP3A4, CYP2C9, and CYP2B6 genetic polymorphisms have also been implicated in HTPRC, but the impact seems small and is debated.^[29] In the present study, genetic testing was not performed to clarify the relationship between HTPRC and hepatosteatosis because such genetic tests cannot be performed in our institution.

Hypertension is a major risk factor for HTPRC. Kim et al.^[27] defined high systolic and diastolic pressures as risk factors for clopidogrel resistance in patients with stable cardiovascular and cerebrovascular diseases. Vascular shear stress, high levels of adhesiveness and aggregability of platelets in patients with hypertension have been suggested as mechanisms for increased HTPRC. DM is another major risk factor for HTPRC, independent of the CYP2C19 genotype.^[30] Insulin inhibits the effect of platelet agonists, such as collagen, ADP, epinephrine, and platelet-activating factor, primarily through the activation of an inhibitory G protein via insulin receptor (IR) substrate 1. Insulin also appears to inhibit the P2Y12 pathway.^[31] However, platelets from patients with type 2 DM

become resistant to the inhibitory effect of insulin, leading to increased P2Y12-mediated suppression of cyclic adenosine monophosphate and decreased antiplatelet drug effects.^[32] In our study, the presence of hypertension and DM were also significantly greater in the HTPRC group ($p < 0.001$). Consistent with earlier research, both hypertension and DM were found to be independent risk factors for HTPRC in our study ($p < 0.001$).

Hepatosteatosis begins with the accumulation of triacylglycerol (TAG) in the liver and is defined as the presence of cytoplasmic lipid droplets in more than 5% of hepatocytes or a TAG level exceeding the 95th percentile for lean, healthy individuals.^[15] An ectopic accumulation of hepatic lipids has clearly been linked to the development of hepatic IR and type 2 DM.^[33] There is a very high prevalence of hepatosteatosis in individuals with IR.^[34] An ultrasonographic study of patients with DM revealed that 127 of 204 diabetic patients displayed fatty infiltration and 87% of the patients with fatty infiltration who consented to a biopsy had a histological confirmation of hepatosteatosis.^[35]

As a result of shared metabolic risk factors, numerous studies have confirmed a strong association between hepatosteatosis and coronary artery disease.^[36] Patients with hepatosteatosis have increased carotid intima media thickness, coronary artery calcification, and endothelial dysfunction.^[37]

Several potential explanations may be posited for the pathophysiological mechanisms through which hepatosteatosis contributes to impaired microvascular flow. First, hepatosteatosis is associated with an increased inflammatory state. C-reactive protein, which is mainly produced by the liver and is a significant marker of adverse cardiovascular outcome, has been shown to be increased in patients with hepatosteatosis.^[38] Second, hepatosteatosis is related to an increased pro-thrombotic state. Fibrinogen and plasminogen activator 1 levels have also been found to be elevated in patients with hepatosteatosis. More importantly, this elevation in inflammatory and pro-coagulant biomarkers has demonstrated a correlation with the degree of hepatosteatosis.^[39] Third, increased endothelial dysfunction in patients with hepatosteatosis may also contribute to impaired myocardial perfusion.^[40] Finally, increased oxidative stress associated with hepatosteatosis may cause microvascular spasm.

Woolsey et al.^[41] reported that hepatic steatosis causes reduced liver CYP3A4 transcriptional activity, in both in vitro and in vivo models of hepatosteatosis. Probable mechanisms for reduced CYP3A4 activity in hepatosteatosis include the effects of inflammation and associated cytokines on hepatic drug metabolism gene expression. CYP3A4 activity inhibition decreases clopidogrel active metabolite concentration. The results of our study indicated that HTPRC was significantly greater in patients with hepatosteatosis and there was a significant association between the grade of hepatosteatosis and the percentage of patients with HTPRC ($p < 0.001$). Hepatosteatosis is known to be more common in diabetics.^[42] However, the results of the present study also demonstrate that hepatosteatosis is an independent risk factor in addition to DM.

Study limitations

This research has some limitations. First of all, a single-center experience may not be an accurate reflection of the whole cohort. Our study consisted of a relatively small patient population size, making the power of the study limited. The results of the analysis cannot be applied to patients with acute coronary syndromes, as only patients with stable coronary artery disease were included. Platelet function analysis was performed using the Multiplate platelet analyzer, whole blood impedance aggregometer which proved to be sensitive for all 3 classes of commonly applied platelet function inhibitors: COX inhibitors (aspirin), ADP receptor antagonists (clopidogrel, prasugrel, cangrelor) and IIB/IIIa antagonists (abciximab, tirofiban, eptifibatide). We only measured HTPRC once in this study; however, some investigators suggest measuring resistance more than once. Multiple assessments may be needed for more accurate results since HTPRC status is liable to change during follow-up. In addition, we defined antiplatelet resistance biochemically, but not clinically. We could not conduct genetic testing for HTPRC due to a lack of foundation. Furthermore, ASA resistance was not studied. There is a possibility it may be associated with HTPRC. We did not follow up with the study patients; long-term follow-up would reveal recurrent ischemic events and more valuable data about the clinical outcomes of patients. Hepatosteatosis was evaluated using ultrasonographic examination and the underlying cause was not fully investigated. In particular, a homeostatic model assessment (fasting blood sugar \times basal insulin level / 405), which is a measure

of IR, was not performed to evaluate the relationship between hepatosteatosis and IR (to identify whether the cause of hepatosteatosis was IR or not). Finally, genetic testing of CYP2C19 and other related genes, such as CYP3A4 and CYP2C9, was not performed to clarify the relationship between HTPRC and hepatosteatosis. This was due to the fact that such genetic tests cannot be performed at our institution.

Conclusion

Despite the shared clinical risk factors and pathophysiological mechanisms, there are no prospective data on an association between HTPRC and hepatosteatosis. Our study is the first study to demonstrate this correlation. In addition, our results indicate that a higher hepatosteatosis grade is strongly associated with HTPRC. This could provide an explanation for the importance of hepatosteatosis screening and treatment in patients with HTPRC.

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