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Research Article

Association of *PAI-1* rs2227631 Variant and Clinical Factors with Recurrent Myocardial Infarction in Patients with Coronary Artery Disease

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Abstract

Background: There is a lack of studies explicating the association between genetic and clinical factors (such as diabetes mellitus) and increased risk of recurrent myocardial infarction (reMI). In this study, the impact of *PAI-1* rs2227631 variant and clinical factors on reMI was analyzed in patients hospitalized due to acute coronary syndromes.

Methods and Results: All patients (n = 674) were hospitalized for percutaneous coronary intervention and stent implantation due to acute coronary syndromes (myocardial infarction or unstable angina). A total of 29.7% of the patients were hospitalized with recurrent MI, which was documented in anamnesis. Genotyping of *PAI-1* rs2227631 was performed for all study patients. Diabetes and *PAI-1* A allele were more frequently found in those patients with reMI who were older than 65 years. Multivariate binary regression analysis revealed that male gender, diabetes and A allele of *PAI-1* rs2227631 increased the odds of reMI infarction by 1.764, 1.708 and 3.373 times, respectively.

Conclusion: Multivariate analysis showed that in aged patients with acute coronary syndromes, the incidence of reMI increased in males, in patients with diabetes and in *PAI-1* rs2227631 A allele carriers.

Keywords: *Diabetes Mellitus; Recurrent MI; PAI-1 rs2227631*

Abbreviations

HUVECs: Human Umbilical Vein Endothelial Cells; MI: Myocardial Infarction; PCI: Percutaneous Coronary Intervention; PAI-1: Plasminogen Activator Inhibitor-1; reMI: Recurrent Myocardial Infarction; SNP: Single Nucleotide Polymorphism; tPA: Tissue Plasminogen Activator; 20-HETE: 20-Hydroxyeicosatetraenoic Acid

Introduction

Coronary heart disease is one of the major causes of morbidity and mortality worldwide [1,2]. Patients who survive acute myocardial infarction (MI) may have a recurrent MI (reMI) after the discharge. ReMI is an event that occurs 28 days or later after the first incident MI and might present in 42% of the patients with MI [3,4]. Patients with reMI usually have worse outcomes than patients with first MI [4-6].

Unhealthy lifestyle [7] and diabetes [5,6] or insulin resistance [8] are among those factors that proved to have a significant impact on incidence of reMI. A total of about 422 million people worldwide had diabetes mellitus in 2014 [9]. In patients with diabetes, circulating levels of plasminogen activator inhibitor-1 (*PAI-1*) increase. Elevated *PAI-1* levels, in turn, lead to the reduction of thrombus lysis [10,11] and subsequent adverse events. In our previous article, we showed that *PAI-1* 4G/5G variant resulted in coronary artery occlusion after MI [12].

PAI-1 is one of the most extensively studied biomarkers of the fibrinolysis system. It plays a crucial role in thrombus lysis and wound healing processes [13]. *PAI-1* controls fibrinolysis and stops the conversion of plasminogen to plasmin [14]. Changes in *PAI-1* expression were observed in the elderly, obese patients, during acute inflammation in diabetic patients and in patients with decreased immune response [15]. Studies also showed that one of the most studied variants of *PAI-1* gene promoter rs2227631 (-844 G/A) was associated with increased *PAI-1* plasma level [16]. Liu., *et al.* suggested that *PAI-1* polymorphism may serve as a genetic biomarker of atherosclerotic diseases [17].

Therefore, we studied the impact of *PAI-1* rs2227631 variant and clinical factors on recurrent MI in patients hospitalized due to acute coronary syndromes.

Methods

Compliance with ethical standards

All the procedures used have been reviewed in compliance with the ethical standards of the Regional Bioethics Committee of Kaunas, Lithuania (the permission number is BE-2-42) and the World Medical Association Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects. Written informed consent was obtained from all the study subjects.

Study population and inclusion criteria

Clinical data and DNA samples were collected from study subjects hospitalized in the Department of Cardiology at the Lithuanian University of Health Sciences (LUHS) in Kaunas, Lithuania, from 2013 to 2017. All of them were hospitalized for PCI and stent implantation due to acute coronary syndromes (myocardial infarction or unstable angina).

Exclusion criteria

Conditions leading to the increased activity of coagulation system: malignant neoplasia, severe inflammation (C-reactive protein level > 100 mg/l or patients following antibiotic therapy due to infection), cardiogenic shock or hypovolemia. The patients who did not consent to take a part in the study were excluded.

Characteristics of the study subjects

A total of n = 674 patients were included into the study. 144 (21.4%) of these patients had diabetes mellitus. Detailed demographic and clinical characteristics are presented in table 1. According to the "Fourth Universal Definition of Myocardial Infarction" MI, which occurred after 28 days following an incident (first) MI, was considered as recurrent MI [18]. A total of a n = 200 patients (29.7%) were hospitalized with recurrent MI (Table 1). Data on the first MI were taken from anamnesis of the patient.

Genotype detection

Genotyping procedures were carried out at the certified Laboratory of Molecular Cardiology, Institute of Cardiology, Lithuanian University of Health Sciences, Kaunas, Lithuania. A standard manufacturer's protocol for rs2227631 TaqMan probes (Applied Biosystems, Waltham, Massachusetts, USA) was used. Genotyping was done on the ABI 7900HT Fast Real-Time PCR Thermocycler (Applied Biosystems, Waltham, Massachusetts, USA) at the Laboratory of Molecular Cardiology, Institute of Cardiology, LUHS.

Variables	Patients with diabetes (n = 144)	Patients without diabetes (n = 530)	p[a] value
Gender n (%)			
Male	97 (67.4)	384 (72.5)	0.253
Female	47 (32.6)	146 (27.5)	
Age n (%)			
< 65 years old male	47 (32.6)	219 (41.3)	0.138
≥ 65 years old male	50 (34.7)	165 (31.1)	
< 65 years old female	9 (6.3)	36 (6.8)	0.553
≥ 65 years old female	38 (26.4)	110 (20.8)	
Age mean ± SD years	67.4 ± 10.31	65.5 ± 11.17	0.066
Smoking n (%)			
Male	26 (18.1)	149 (28.1)	0.221
Female	0	13 (2.5)	
Incident MI suffered n (%)			
First	90 (62.5)	384 (72.5)	0.024
Recurrent MI[b]	54 (37.5)	146 (27.5)	

Table 1: *Demographic and clinical characteristics of studied patients.*

[a]: Fisher exact test; [b]: Acute myocardial infarction occured after the 28 days of index MI.

Abbreviations: MI: Myocardial Infarction; ST elevation - Myocardial Infarction.

Statistical analysis

Frequencies of *PAI-1* rs2227631 variants are presented in percentage. χ^2 analysis test was used to determine the deviation of allele distribution from the Hardy-Weinberg equilibrium. Fisher's exact test and Pearson χ^2 analysis were used for categorical variables. A binary logistic regression model was used to identify independently associated clinical and genetic factors, which determine reMI. All variables were chosen for the multivariable model by backward selection, and in the final model, only those with $p < 0.05$ were left.

Results

Patients with diabetes more frequently had recurrent MI as compared to non-diabetic patients (Table 1). Also, *PAI-1* rs2227631 A allele (Table 2) was more frequent in the patients with recurrent MI.

PAI-1 rs2227631 polymorphism genotypes and alleles	Patients with recurrent MI n (%) (n = 200)	Patients without recurrent MI n (%) (n = 474)	Pearson χ^2, p value
AA	84 (42)	172 (36.3)	6.498, $p = 0.039$
AG	96 (48)	219 (46.2)	
GG	20 (10)	83 (17.5)	
A allele	260 (65.6)	563 (59.4)	4.623, $p = 0.031$
G allele	136 (34.4)	385 (40.6)	

Table 2: *Genotype and allele distributions of the rs2227631 variant in patients according to recurrent MI.*

The distribution of the *PAI-1* rs2227631 polymorphism genotypes and alleles of the studied sample matched the Hardy-Weinberg equilibrium (p = 0.708 and p = 0.613, respectively).

Distribution of rs2227631 genotypes in patients with diabetes according to age groups

A detailed analysis revealed that diabetes (Table 3) and A allele (Table 4) were more frequently found only in those patients with recurrent MI who were older than 65 years. No significant differences were determined in younger patients.

Patients age group	Variable	Recurrent MI n (%) (n = 200)	No recurrent MI n (%) (n = 474)	Pearson χ^2, p value
Older than 65 years	Patients with diabetes n (%)	39 (32.2)	49 (20.2)	6.307, p = 0.014
	Patients without diabetes n (%)	82 (67.8)	193 (79.8)	
Younger than 65 years	Patients with diabetes n (%)	15 (19)	41 (17.7)	0.069, p = 0.8
	Patients without diabetes n (%)	64 (81)	191 (82.3)	

Table 3: *Prevalence of diabetes in patients with recurrent MI.*

Patients age group	rs2227631 polymorphism genotypes and alleles	Patients with recurrent MI n (%) (n = 200)	Patients without recurrent MI n (%) (n = 474)	Pearson χ^2, p value
Older than 65 years	AA	54 (44.6)	83 (34.3)	11.568, p = 0.003
	AG	59 (48.8)	111 (45.9)	
	GG	8 (6.6)	48 (19.8)	
	A allele	183 (70.9)	277 (57.2)	13.403, p < 0.001
	G allele	75 (29.1)	207 (42.8)	
Younger than 65 years	AA	30 (38)	89 (38.4)	0.0037, p = 0.99
	AG	37 (46.8)	108 (46.6)	
	GG	12 (15.2)	35 (15)	
	A allele	97 (61.4)	286 (61.6)	0.003, p = 0.96
	G allele	61 (38.6)	178 (38.4)	

Table 4: *Genotype and allele distributions of the rs2227631 variant in patients with recurrent MI according to the age groups.*

Multivariate binary regression model for recurrent MI in 65 years and older patients

Multivariate binary regression analysis (Table 5) showed that male gender, diabetes and rs2227631 AA or AG variants increased the odds of recurrent MI by 1.764, 1.708 and 3.373 times, respectively.

Variable	OR	95% CI	p value
Gender (male)	1.764	1.106 - 2.815	0.017
Diabetes mellitus	1.708	1.029 - 2.834	0.038
rs2227631 AA+AG versus GG	3.373	1.523 - 7.472	0.003

Table 5: *Factors determining recurrent MI in 65 years and older patients.*

Abbreviations: OR: Odds Ratio; CI: Confidence Interval.

Discussion

This is the first study, which showed that *PAI-1* rs2227631 variants were associated with recurrent myocardial infarction in aged patients. In addition, a multivariate analysis model showed that male gender, diabetes and rs2227631 A allele increased the odds of recurrent MI.

Ageing is responsible for impairment of different organ systems. Elderly patients have coronary vascular disease risk factors such as diabetes, metabolic disease and hypertension more frequently than younger patients [19-21]. A study representing two groups of subjects, healthy centenarians (age range from 100 to 105 years) and aged persons (age range from 62 to 90), showed that plasma *PAI-1* activity correlates with insulin resistance, fasting plasma triglycerides and the age of the studied aged subjects. It is noteworthy that healthy centenarians had a higher *PAI-1* activity, but they also had lower insulin resistance than the aged subjects [22]. Our results also show that elderly patients with recurrent MI more frequently had diabetes. It was already proved by Jin., *et al.* that fasting blood glucose (FBG) level might be associated with subsequent MI risk in non-diabetic patients [23]. Moreover, decrease in FBG level was associated with lower risk of MI in their study [23]. Recent findings indicate that blood glucose stimulates production of arachidonic acid metabolite - 20-hydroxyeicosatetraenoic acid (20-HETE), which, in turn, stimulates insulin secretion in pancreatic β-cells [24]. Previous research also confirmed the impact of 20-HETE on the development of hypertension and vascular dysfunction [25,26]. Results of Tunaru., *et al.* [24] explain in more detail the function of 20-HETE during diabetes. The authors show that patients with type 2 diabetes mellitus have lower 20-HETE concentrations [24]. This may lead to endothelial dysfunction in diabetes and other conditions related to insulin resistance [25]. 20-HETE inhibits insulin-stimulated NOS function and production of NO in human umbilical vein endothelial cells (HUVECs) [25]. Thus, glucose intolerance and cardiovascular disease are related, and this is usually manifested with the age of the patient.

The rs2227631 (-844 A) variant is associated with the increased *PAI-1* plasma level and lower activity of fibrinolysis system [16]. To date, little data are available on the relation between rs2227631 and coronary heart disease. Abboud., *et al*. [16] showed that the risk of MI was higher in rs2227631 -844A carriers. They also had elevated plasma *PAI-1* and reduced tPA levels [16]. Another study conducted by Su., *et al.* [27] showed that rs2227631 A allele increased the risk of coronary heart disease among nonsmokers in Han Chinese population [27]. Finally, -844A>G (rs2227631) is in a strong linkage disequilibrium (LD) with the -675 4G/5G variant (SNP rs1799889) [28]. These two variants are tag SNP's and have a similar effect on the *PAI-1* activity [29]. *PAI-1* gene promoter in 5G variant carrying subjects binds a nuclear protein that acts as a transcriptional repressor. Thus, a higher *PAI-1* activity is usually detected in 4G carriers [29]. Results from our previous study showed that patients with 4G/5G variant more frequently had coronary artery occlusion after myocardial infarction [12]. *PAI-1* 4G/4G was detected in 32.4%, 4G/5G - in 49.5% and 5G/5G - in 18.1% of the studied patients [12]. Results from this study show similar distribution of *PAI-1* rs2227631 variants (Table 6). *PAI-1* rs2227631 A allele, which determines higher *PAI-1* activity, was more frequent in our studied patients with recurrent MI.

The frequencies of *PAI-1* rs2227631 differ among countries (Table 6). We were only able to find two studies carried out in the neighboring countries: one describes patients from Latvia following cardiac surgery [30]. Another one describes Finnish cohort of the patients with coronary heart disease [31]. Both studies showed lower prevalence of the rs2227631 (-844G>A) AA variant as compared to our patients' sample. The similar distribution of rs2227631 variants as compared to our patients' sample was observed in healthy Canadian women studied by Bentov., *et al* [32]. The lowest frequency of AA variant was observed in table 6 Mexican [33] and Asian populations [34,35].

This study also draws attention to the fact that rs2227631 A allele was more prevalent only in elderly patients with recurrent MI. No such effect was observed in younger patients. Prolongation of thrombus lysis progresses with the patient's age [11] and is usually aggravated in such conditions as insulin resistance, atherosclerosis or inflammation [36]. McMillin*., et al*. 2014 [37], confirmed that *PAI-1*

expression is linked to the age of the patient, abdominal fat and resistance to insulin [37]. There is another mechanism that may explain the impact of age on *PAI-1* activity. It is hypothesized that the activity of P53 (product of *TP53*), which may regulate the concentration of *PAI-1*, is age and insulin-dependent [38].

Based on the multivariate analysis model, which reveals that such factors as male gender, diabetes and A allele of *PAI-1* rs2227631 increase the odds of recurrent MI in 65 years or older patients, these data may help to identify patients at high risk of reMI.

Conclusion

Multivariate analysis model showed that in aged patients with acute coronary syndromes, the incidence of reMI increased in males, in patients with diabetes and in *PAI-1* rs2227631 A allele carriers.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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