

Frequency of Factor V Leiden Mutation in Dilated Cardiomyopathy Patients with Left Ventricular Thrombus

SOL VENTRİKÜL TROMBÜSÜ OLAN DİLATE KARDİYOMİYOPATİLİ OLGULARDA FAKTÖR V LEİDEN MUTASYONUNUN SIKLIĞI

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Abstract

Objective: Activated protein C resistance was demonstrated as an independent risk factor for left ventricular (LV) thrombus in patients with dilated cardiomyopathy. However, factor V Leiden mutation (FVLM), which is mainly responsible for activated protein C resistance, has not been investigated yet. In this study, our goal was to find out whether FVLM is a risk factor for left ventricular thrombi in these patients.

Material and Methods: Sixty-one patients with idiopathic dilated cardiomyopathy who had ejection fractions $\leq 40\%$ and LV end-diastolic diameters >6.0 cm were divided into two groups according to 2-dimensional transthoracic echocardiographic examination; Group I (n= 23) with left ventricular thrombus and Group II (n= 38) without LV thrombus. These groups were compared for clinical, echocardiographic and hematologic parameters. FVLM was analyzed using the polymerase chain reaction.

Results: The prevalence of FVLM was 9% (2/23) in patients with LV thrombus, and 8% (3/38) in patients without left ventricular thrombus ($p > 0.05$). No significant differences in fibrinogen concentration, tissue plasminogen activator, von Willebrand factor and plasminogen activator inhibitor-I levels were found in patients with LV thrombus compared to with those without LV thrombus ($p > 0.05$). None of the patients had protein S and antithrombin deficiency. Left ventricular ejection fraction was lower and left ventricular end diastolic diameter was higher in Group I than in Group II ($p < 0.05$). Multivariate regression analysis to identify variables associated with left ventricular thrombosis showed that LV ejection fraction and LV end diastolic diameter were independent predictors of LV thrombus ($p < 0.05$).

Conclusions: In the light of these findings, it is possible to conclude that FVLM is not a risk factor for left ventricular thrombus formation in dilated cardiomyopathy.

Key Words: Cardiomyopathy, factor V Leiden, left ventricular thrombus

Özet

Amaç: Aktive protein C rezistansının dilate kardiyomyopatili olgularda sol ventrikül (LV) trombüs gelişimi için risk faktörü olduğu bilinmektedir. Bununla beraber dilate kardiyomyopatide LV trombüsü gelişiminin aktive protein C rezistansının %85'inden sorumlu olan faktör V Leiden mutasyonu (FVLM) ile ilişkili olup olmadığı araştırılmamıştır. Bu çalışmada FVLM'nin dilate kardiyomyopatili hastalarda LV trombüs gelişimi için risk faktörü olup olmadığı araştırıldı.

Gereç ve Yöntemler: LV diyastol sonu çapı >6.0 cm ve ejeksiyon fraksiyonu $\leq 40\%$ olan 61 idiyopatik dilate kardiyomyopatili hasta; 2 boyutlu transtorasik ekokardiyografide LV'de trombüs saptanan (Grup I= 23 olgu) ve saptanmayan (Grup II= 38 olgu) olmak üzere iki gruba ayrıldı. Olguların klinik ve ekokardiyografik verileri karşılaştırıldı. FVLM polimeraz zincir reaksiyonu kullanılarak saptandı.

Bulgular: FVLM sıklığı LV trombüs olanlarda %9 (2/23), trombüs olmayanlarda %8 (3/38) idi. İki grupta fibrinojen, doku plazminojen aktivatör, von Willebrand faktör ve plazminojen aktivatör inhibitör-1 seviyeleri arasında anlamlı fark yoktu ($p > 0.05$). Hiç bir olguda protein C ve antitrombin defektine rastlanmadı. LV ejeksiyon fraksiyonu ve LV diyastol sonu çapı grup I'de anlamlı derecede daha yüksekti. Çoklu regresyon analizinde LV diyastol sonu çap ve ejeksiyon fraksiyonunun LV trombüsü için bağımsız risk faktörleri olduğu görüldü.

Sonuç: Bu çalışma FVLM'nun dilate kardiyomyopatili olgularda LV trombüs gelişimi için bir risk faktörü olmadığını düşündürmektedir.

Anahtar Kelimeler: Kardiyomyopati, faktör V Leiden, sol ventrikül trombüsü

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Dilated cardiomyopathy (DCM) is associated with an increased risk of thromboembolic events, which may be related to a prothrombotic or hypercoagulable state.¹⁻³ The

clinical importance of left ventricular thrombus lies in its potential to embolize. Reports indicate that left ventricular (LV) thrombus formation in patients with global or segmentary LV systolic dysfunction is associated with reduced blood flow velocity and stasis, the presence of abnormal endocardial surfaces, atrial fibrillation, LV wall motion abnormalities and coagulation disturbances.⁴⁻⁹ Knowledge on the hematological mechanism causing thrombosis has grown enormously in recent years. The genetic defect of coagulation known as factor V Leiden mutation (FVLM) produces a resistance to degradation by activated protein C (APC), and reduces the anticoagulant effect of APC. Resistance to APC was shown to be an important hereditary risk factor for the development of thromboembolic disorders.¹⁰⁻¹³ More than 85% of patients with thrombus embolic disorders has been identify as having the factor V Leiden mutation.³ The role of FVLM in venous thrombosis and unexplained arterial thromboembolic events was established.^{14,15} The relationship between FVLM and thrombus formation in LV with DCM has not been investigated until now, to the best of our knowledge.

Therefore, the aim of this study was to investigate whether FVLM is a risk factor for the formation of LV thrombus in patients with DCM.

Material and Methods

Study Population

From July 2000 to January 2004, 90 patients with the diagnosis of LV dilation and dysfunction documented by echocardiography or left ventriculography, who were either admitted to the hospital or observed in our outpatient clinic, were prospectively enrolled in this study. Inclusion criteria were LV end diastolic diameter >6 cm and LV ejection fraction <40%. Twenty-nine patients were excluded from the study for various reasons; 7 patients (7.7%) had taken oral anticoagulants, 18 (20%) patients had coronary bypass surgery and 4 (4.4%) patients had poor two-dimensional echocardiographic image quality. The remaining 61 patients (35 males, 26 females, mean age 55 ± 10)

were enrolled in the study. Informed consent was obtained from all subjects after a full explanation of the study. This study was approved by Hospital Ethics Committee.

Coronary angiography was performed in all patients. The patients with coronary atherosclerosis according to angiographic results were considered as ischemic cardiomyopathy group. The patients with global hypokinesia and normal coronary angiograms were classified as non-ischemic DCM. In the presence of a normal coronary angiogram with segmentary wall motion abnormalities related to scar and/or ischemia detected by myocardial perfusion sintigraphy, LV dysfunction was classified as ischemic cardiomyopathy.

Echocardiographic studies

A complete 2-dimensional and pulse Doppler echocardiographic examination was performed using an Aloka SSD 5000 ultrasound (Aloka Inc, Tokyo, Japan) machine with a 3.5-MHz transducer and Vingmed CFM 725 ultrasound machine (Horten, Norway) with a 3.25-MHz transducer. The diagnosis of LV thrombus was defined by the presence of an echogenic mass with a margin distinct from the LV wall and was visible throughout the cardiac cycle in at least two different echocardiographic views.¹⁶⁻¹⁸ M-mode LV echocardiographic measurements were performed by parasternal long axis views according to the American Society of Echocardiography recommendations.¹⁹ LV end-diastolic and end-systolic volumes were determined by apical two- and four-chamber views by using the Simpson biplane formula according to the recommendations of the American Society of Echocardiography.²⁰ Ejection fraction was calculated as (End-diastolic-End-systolic volume)/End-diastolic volume. To calculate the wall motion score index (WMSI), the left ventricle was divided into 16 segments. Segmental wall motion was graded as previously described.¹⁹ The WMSI was calculated by summation of individual segment scores divided by the number of interpreted segments. A physician who was blinded to the patients' clinical and laboratory data interpreted the echocardiographic studies.

Blood sampling and assay

Blood samples were drawn from two groups in fasting state and were collected in EDTA-tubes (Vacutaine, Becton Dickinson, Meylon, France). The tubes were centrifuged at 2000 g for 20 minutes. DNA was extracted from blood using a nucleospin DNA extraction kit (Macherey-Nagel GmbH, Düren, Germany). DNA concentrations were measured spectrophotometrically at 260 nm and stored at -7°C. All measurements were subsequently performed within 3 weeks of collection. Ten microliters of DNA was amplified by polymerase chain reaction (PCR) in a Techne-Genius thermocycler (Techne-Cambridge, UK) followed by digestion with the Mnl I restriction enzyme (New England Biolabs) at 37°C for 16 hours. The products were then subjected to metaphor agarose gel electrophoresis. After completion of electrophoresis, the gels were photographed under ultraviolet transillumination. The technicians were blinded to the a specimen, which was collected from cases.

The samples were collected, centrifuged, and stored in appropriate tubes and refrigerated until testing. Plasma concentrations of fibrinogen were measured by using a Micro-capillary assay. Quantitative determination of protein C was performed by Staclot® protein C kits based on the inhibition of factor Va (Diagnostica Stago®, Asnières, France) and a ratio less than 65% was considered to reflect deficiency of protein C. Antithrombin measurement was performed by the synthetic chromogenic substrate method and Stacrome AT III kits (Diagnostica Stago® Asnières, France) and a ratio less than 80% was considered to reflect AT III deficiency. The tissue plasminogen activator (t-PA), von Willebrand factor (vWF), and plasminogen activator inhibitor-I (PAI-1) were measured with enzyme-linked immunosorbent assay according to the manufacturer’s instructions (Diagnostica Stago®, STA Compact France).

Statistical analysis

Results are expressed as the mean ± SD or percentages. Comparisons between the two groups were performed by means of an unpaired t-test for

continuous variables. Categorical variables were analyzed with contingency tables using the Chi-square test. Multivariate logistic regression analysis was performed to identify independent predictors of LV thrombus. Plasma fibrinogen, vWF, PAI-1 and t-PA levels, presence of hypertension, diabetes mellitus and smoking, and ejection fraction and left ventricular end-diastolic diameters were checked with multivariate analysis. A p value <0.05 was considered statistically significant.

Results

LV thrombus was detected in 23 (37.7%) of 61 patients with DCM. Patients were assigned to two groups; Group 1 consisted of 23 patients with LV thrombus (14 men, 9 women; aged 45-79 years, mean 59 ± 8 years) and Group 2 consisted of 38 patients (21 men, 17 women; aged 41-78 years, mean 60±8 years) without LV thrombus.

Patients’ baseline characteristics are shown in Table 1. There were no significant differences in age, gender, current therapy and risk factors for atherosclerosis between the two groups. None of the patients had protein C and antithrombin deficiency. Levels of fibrinogen, t-PA, vWF, and PAI-1 did not differ significantly in patients with LV thrombus compared with those without LV thrombus (Table 2).

Ischemic DCM was detected in 36 patients (59%). The rate of ischemic DCM was not different between Group 1 (in 13 of 23 patients; 56.5%)

Table 1. Baseline characteristics of the study participants.

	Group I (n= 23)	Group II (n= 38)	p value
Age, years	0.160	59 ± 8	60 ± 8
Sex, % male	60	55	0.120
Diabetes, (%)	26	27	0.513
Hypertension, (%)	39	31	0.256
Family history, (%)	46	53	0.324
Smoking, (%)	43	38	0.347
Ischemic DCMP, n (%)	13 (56)	23 (61)	0.224
ASA, (%)	65	69	0.345
Beta-blocker, (%)	35	40	0.124
ACE inhibitor, (%)	58	61	0.334
Diuretic (%)	45	53	0.241

Abbreviations: ACE= angiotensin-converting enzymes, ASA= aasetil salicylic asit. DCMP= dilated cardiomyopathy

Table 2. Comparison of factor V Leiden mutation, echocardiographic characteristic and other laboratory parameters in patients with and without left ventricular thrombus.

	Thrombus (n=23)	No thrombus (n=38)	p Value
Cholesterol, mg/dl	176 ± 25	184 ± 32	0.241
HDL-cholesterol, mg/dl	34 ± 7	37 ± 8	0.134
LDL-cholesterol, mg/dl	114 ± 6	121 ± 11	0.256
Fibrinogen, mg/dl	316 ± 64	365 ± 124	0.241
Factor V Leiden mutation, (%)	9	8	0.127
t-PA, ng/dl	16.2 ± 6.4	15 ± 12.47	0.82
PAI-1, ng/dl	24.5 ± 8.4	27.7 ± 10.4	0.72
Von Willebrand factor, (%)	116 ± 14	105 ± 12	0.63
Left ventricular WMSI	2.1 ± 0.90	1.9 ± 0.72	0.346
LVESD, cm	5.3 ± 0,5	5,2 ± 0,3	0.178
LVEDD, cm	7.1 ± 0.7	6.8 ± 0.4	0.003
EF, (%)	31 ± 5	35 ± 4	0.001

Abbreviations: EF= ejection fraction HDL= high-density lipoprotein, LDL= low-density lipoprotein, WMSI= wall motion score index, LVESD= left ventricular end-systolic diameter, LVEDD= left ventricular end-diastolic diameter, PAI-1=plasminogen activator inhibitor, t-PA= tissue plasminogen activator.

and Group 2 (in 23 of 38 patients; 60.5%) (p> 0.05). The echocardiographic data of the groups are presented in Table 2. LV end diastolic dimension and LV ejection fraction were significantly higher in Group I than in Group II. Except these, other parameters were not significantly different between the groups.

Factor V Leiden mutation

The frequencies of FVLM in both groups are shown in Table II. The prevalence of the FVLM was 9% (2/23) in patients with thrombus, and 8% (3/38) in patients without thrombus (p> 0.05). Incidence of FVLM was similar in patients with ischemic cardiomyopathy and non-ischemic cardiomyopathy (p> 0.05).

Univariate analysis that was performed by enrolling the clinical and echocardiographic factors showed that LVEDD and decreased LV EF were related to LV thrombus. In multivariate logistic regression analysis, which was performed to assess the factors that were independently related to LV thrombus formation, LV end diastolic dimension and LV ejection fraction were independent predictors of LV thrombus formation (p= 0.008 and p= 0.02, respectively).

Discussion

A number of reports indicate that FVLM is associated with venous and arterial thromboembolic events but it is not related to LV thrombus formation in patients with acute myocardial infarction.^{11,12,15,21,22} However, to the best our knowledge no study reported a relation between FVLM and formation of LV thrombus in patients with DCM. This is the first study that investigates the relation between FVLM and LV thrombus in patients with DCM.

FVLM has recently emerged as an important cause of thrombosis, and it is associated with a single point mutation in the factor V gene (FVQ 506 or factor V Leiden), which removes an important cleavage site for activated protein C. The FVLM is the most commonly described congenital abnormality predisposing to the development of familial thrombophilia and shows significant variable prevalence among different ethnic groups and countries.^{11,22,24} FVLM is present in 1% to 7.3% of the general population.^{11,24,25} In the present study, we found that the prevalence of FVLM was 9% and 8% in patients with and without LV thrombus respectively. Differences in FVLM prevalence between previous studies and our study may be related to variations in geographic regions, ethnic origins and to the limited number of our patients.

In a recently published study, Erbay et al reported that activated protein C resistance was an independent predictor of LV thrombus in patients with DCM.²⁶ However, FVLM was not a predictor for LV thrombus in patients with DCM in our study. Although FVLM is a cause for activated protein C resistance, it is not entirely responsible from this condition. FVLM is found in 85-90% of individuals with activated protein C resistance.^{22,23} The results of our and Erbay's study suggest that other causes of activated protein C resistance are related to LV thrombus rather than FVLM.

The documented incidence of LV thrombus varies between 11% and 44% in patients with LV dysfunction and increases up to 75% in patients with DCM.^{1-3,18,27} A poorly contracting ventricle allows blood stasis, which can lead to thrombus formation.²⁸ A number of echocardiographic stud-

ies suggest that abnormal flow patterns within the left ventricle, lower EF, decreased fractional shortening, increased LVEDD, and mitral regurgitation severity may also be associated with LV thrombus formation in patients with DCM.^{8,27-31} Additionally, ischemic etiology of the dilated cardiomyopathy and increased platelet activation were found to be independent predictors of thrombus formation in DCM.^{30,32} In our study, LV end diastolic diameter and lower EF were predictors of thrombus in patients with DCM. These findings are consistent with previous studies.²⁷⁻³⁰

Study Limitations

The most important limitation of our study was the small number of patients studied. The activated protein C resistance was not assessed, which is another limitation of the present study. Large prospective studies are needed to establish whether FVLM is a risk factor for LV thrombus formation in patients with DCM.

In conclusion, it is possible to say that FVLM is not a risk factor for LV thrombus formation in dilated cardiomyopathy.

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