Resistance to Activated Protein C and FV Leiden Mutation in Patients With a History of Acute Myocardial Infarction or Primary Hypertension


This study was designed to investigate both resistance to activated protein C (APC-R) and the factor FV Q506 mutation incidence in patients with a history of acute myocardial infarction (AMI) and patients with primary hypertension (PH), a high-risk group for arterial thrombosis. Eighty patients with a history of AMI (group A), 160 patients with a history of PH (group B), and 124 age-matched controls without arterial disease (group C) were studied. APC-R was determined using the Coatest APC Resistance Kit of Chromagenix, Sweden. The prevalence of the FV Q506 mutation was estimated by DNA analysis (Bertina method). The prevalence of the FV Q506 mutation was 20%, 13.75%, and 8% in groups A, B, and C, respectively (A vs C P = .0466). The prevalence of APC-R was 47.5% in group A vs 13% in group C (P < .0001) and 36.25% in group B vs 13% in group C (P < .0001). The response to activated protein C expressed as mean value ± SD was 2.05 ± 0.33 in group A vs 2.56 ± 0.46 in group C (P < .05) and 2 ± 0.22 in group B vs 2.56 ± 0.46 in group C (P < .05). These findings suggest that patients with a history of AMI or PH have a significantly increased incidence of both APC-R and FV Q506 mutation compared with the control group. These findings support the hypothesis that these anticoagulant defects may be risk factors for arterial thrombosis. Am J Hypertens 2000;13:61–65 © 2000 American Journal of Hypertension, Ltd.

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Reduced response to activated protein C (APC-R), an inherited hemostatic defect,1 has been identified recently as a risk factor for venous thrombosis.2,3 Depending on the selection criteria, resistance to APC has been found in 18 to 50% of thrombotic patients4 and has a prevalence of 3 to 5% in the general population.3 In more than 90% of the patients, the APC-R is caused by a single point mutation in the gene of factor V (G to A at nucleotide position 1691), which predicts replacement of Arg (R)506 in the APC cleavage site with a Gln (Q).4–6 After activation by thrombin factor Xa, mutated factor V (FV Q506) is less efficiently degraded by APC than normal factor V (FV R506), which results in increased thrombin generation and a hypercoagulable state.4 Heterozygosity for the factor V gene defect is associated with a 5- to 10-fold increased risk of thrombosis.3,4 However, this “hypercoagulant” state may
never be “expressed” unless the carriers of the defect are expressed to other risk factors. Numerous studies showed a strong correlation between APC-R, FV Q506 mutation, and venous thrombosis, whereas the relationship between these defects and the arterial thrombosis (coronary or cerebrovascular) are not clear and the initial results of the clinical studies are controversial. The aim of our study was to estimate the prevalence in patients with a history of acute myocardial infarction (AMI), that is, established arterial thrombosis, in patients with primary hypertension, that is, a high-risk group for arterial thrombosis, and to compare the findings to those of healthy subjects without arterial disease. It must be pointed out that there are no previous published data about the prevalence of these anticoagulant defects in hypertensives.

MATERIALS AND METHODS

Eighty patients with a history of myocardial infarction (30% women; mean age, 55.4 ± 5.2 years; group A), who were hospitalized in our general hospital, 160 patients with a history of primary hypertension under treatment (34% women; mean age, 56.2 ± 4.8 years; group B), and 124 healthy controls (36% women; mean age, 55.8 ± 4.65 years, without arterial disease; group C) were studied. Both patients and control subjects were born in Greece and were living in the Athens region. Clinical characteristics of the study population are shown in Table 1. Two of the three characteristics of typical chest pain, a transient increase in cardiac enzymes to more than twice the upper limit and electrocardiographic changes typical for myocardial infarction had to be present in the discharge report or hospital record of a patient with MI. Primary hypertension diagnosis was based on the JNC-VI criteria. The control group consisted of healthy individuals without a history of myocardial infarction, ischemic cerebrovascular disease, or venous or pulmonary thrombosis, with a normal surface electrocardiogram, normal heart ultrasound-Doppler, and negative exercise test (Bruce protocol). Patients using anticoagulant drugs or having a history of venous or pulmonary thrombosis were excluded.

APC-R Determination Blood sampling was performed in fasting subjects after a 20-min period of rest in supine position, between 8 and 9 AM. Peripheral blood was collected in vacutainer tubes containing 0.5 mL of 0.12 mol/L sodium citrate (9:1, vol:vol) and centrifuged at 2000g for 20 min at room temperature to obtain platelet-poor plasma. The anticoagulant response of the patient’s plasma to APC was determined using the Coatest APC Resistance Kit (Chromagenix, Mölydal, Sweden) on a Stago STA coagulometer (Stago Diagnostic, Asnieves sur Seine, France). This APC-R assay uses APC, synthetic phospholipids, calcium chloride, and colloidal silica as an activator in an activated partial thromboplastin time (APTT)-based assay. Results are expressed as the ratio of the APTT clotting time of the plasma in the presence of exogenous APC to the APTT clotting time without exogenous APC. When a reduced response to APC was determined, a second sample from the same patient was examined (laboratory normal values of APC sensitivity ratio, ≥1.93).

Gene Analysis The FV Q506 mutation was detected as described by Bertina et al. DNA was isolated from stored leukocytes. A genomic fragment (267 nucleotide bases in size) containing the nucleotide sequence coding for arginine-506 (R506) was amplified by polymerase chain reaction. The amplified genomic fragment was digested with the restriction endonuclease MnlI. Digestion of the normal FV arginine-506 gene segment yielded three fragments of 37, 67, and 163 bases, whereas digestion of the mutated FV glutamine-506 (Q506) yielded two fragments of 67 and 200 bases. These fragments were identified by agarose gel electrophoresis and analysis was performed on two separate occasions.

Statistical Analysis Results are expressed as mean value ± 1 standard deviation; n indicates the number of patients. The incidence of the disturbance appear-
 ance is expressed as a percentage from baseline. Intergroup comparisons of continuous variables were performed using analysis of variance supplemented by Sheffé’s test, whereas intergroup comparisons of discrete variables were performed with contingency tables. A P value <.05 was considered statistically significant.

RESULTS

The genetic analysis for the factor V missense mutation showed the following findings. The incidence of the mutation was 20% in group A (16 of 80 patients with myocardial infarction), 13.75% in group B (22 of 160 patients with primary hypertension), and 8% in group C (10 of 124 healthy controls, group A \( v \) C \( P = .0466 \), A \( v \) B \( P = \) NS, B \( v \) C \( P = \) NS). The incidence of reduced response to APC (APC sensitivity ratio, 1.93) was 47.5% in group A (38 of 80 patients with myocardial infarction) \( v \) 13% in group C (16 of 124 healthy controls, group A \( v \) C \( P = .0001 \), A \( v \) B \( P = \) NS, B \( v \) C \( P = NS \)).

The response to activated protein C expressed as mean value ± SD was 2.05 ± 0.33 in group A \( v \) 2.56 ± 0.46 in group C (\( P < .05 \)) and 2 ± 0.22 in group B \( v \) 2.56 ± 0.46 in group C (\( P < .05 \)). There was no statistically significant difference between groups A and B (2.05 ± 0.33 \( v \) 2 ± 0.22, \( P = \) NS).

DISCUSSION

In early 1993, using a simple assay based on the poor anticoagulant response to exogenous APC in an APTT assay system, Dahlbäck et al.\(^1\) reported the identification of a new familial defect in the protein C anticoagulant pathway of hemostasis (APC-R). In cohorts of thrombosis patients, APC-R is found in 30% to 50% of patients \( v \) 0% to 7% in the control group.\(^2,3,9,10,16\) Differences in frequencies are related to differences in selection criteria for patient or in population prevalence of APC-R.

In more than 90% of the patients, the APC-R is caused by a single point mutation in the gene for factor V (G to A at nucleotide position 1691), which predicts replacement of arginine (R)506 in the APC cleavage site with a glutamine (Q).\(^4-6\) As a consequence of this disturbance, mutated factor V (FV Q506) is less efficiently degraded by APC than normal factor V (FV R506), which results in increased thrombin generation and a hypercoagulable state.\(^3\) The factor V gene mutation is highly prevalent in the general population; approximately 2% to 4% in both the Netherlands\(^4\) and England\(^17\) studies and 8% in the Greece\(^18,19\) studies, making this hereditary abnormality of the clotting system the most common one. Several studies have demonstrated an association between APC-R and FV Q506 mutation (factor V Leiden) and venous thrombosis or embolism.\(^2,3,9,10,12\) Whether factor V Leiden influences the risk of arterial disease is arguable, and few studies have investigated the asso-
Greece is twofold compared to other European countries.20–22 More recently, Rosendaal et al23 concluded that factor V Leiden increases the risk of MI in women, indicating that the Leiden mutation is a significant risk factor for early MI but only if another risk factor is present, particularly smoking.24

In our study, we examined the incidence of APC-R and the FV Q506 mutation in three population groups. Group A consisted of patients with established arterial thrombosis (ie, survivors of an AMI), group B consisted of patients at high risk for cardiovascular events as are those with established primary hypertension, and group C consisted of healthy subjects with no history of arterial or venous thrombosis. The percentage of the FV Q506 mutation in the control group (group C) was as low as in the general population.18,19

The prevalence of the mutation in the group of patients with MI (group A) was 20% (16 of 80 patients). This high percentage is in accordance with the data reported by März et al11 and by Rosendaal et al23 (11% and 12%, respectively in patients with a history of coronary artery disease and MI), but differs from the data of Samani et al.13 We must emphasize here that the incidence of FV Q506 in the general population in Greece is twofold compared to other European countries.18,19 In a recent study, Rosendaal et al23 reported that factor V Leiden mutation was found more often in women with MI (10%) than among controls (4%) and the risk was increased fourfold (1.2 to 12.1%) when adjusted for major cardiovascular risk factors. Moreover, Doggan et al25 concluded that genetic variations in thrombotic risk factors, such as prothrombin 20210A and factor V Leiden mutation increase the risk of MI in men especially when other cardiovascular risk factors are present as well. It is obvious from these published data that the question remains open. Given the high frequency of FV Q506 in the general population and the high prevalence of cardiovascular risk factors compared to other genetic mutations relevant to thrombosis, it seems that further investigation is necessary to confirm the hypothesis that the FV Q506 mutation is a risk factor for AMI. Until we have enough data we must treat the known risk factors for cardiovascular disease as aggressively as possible.

The incidence of the FV Q506 mutation in the group of patients with primary hypertension (group B) was 13.75% (22 of 160 patients). There are no published data in the international literature concerning the incidence of the mutation in the hypertensive population; this is the first report. The follow-up of these patients may reveal an association between this mutation and cardiovascular complications in this group of patients.

APC-R prevalence in the group of patients with previous MI (group A) was 47.5%, this is in accordance with the data reported in patients with venous thrombosis.3 From the literature it is known that APC-R is associated with ischemic cerebrovascular episodes, but not with AMI.25 In our study the incidence of APC-R was extremely high (~50% of the study population with previous MI). The prevalence of the APC-R in the group of hypertensives (group B) was also increased (36.25%). In a recent study, Rozand et al27 reported the incidence of APC-R among patients with arterial thrombosis or venous thrombosis equal to 18%, but there are no data concerning hypertension. The prevalence of APC-R in the control group of patients was 13%, more than double the control group of patients with venous thrombosis.23,16 The mean value of APC-R in group A was 2.05 ± 0.33 v 2.56 ± 0.46 in group C, P < .05, whereas APC-R in group B was 2 ± 0.22 v 2.56 ± 0.46 in group C, P < .05. Although there was no significant difference between groups A and B (MI and primary hypertension, respectively), the APC sensitivity ratio in our laboratory is ≥1.93 and in other studies, ≥2.0 or ≥2.35 for the control group, making our criteria stricter.

In conclusion, the results of the present study show that genetic variations in thrombotic risk factors, such as FV Q506 mutation and APC-R, have a significantly higher incidence in patients with previous MI or primary hypertension compared to controls. These results are the first concerning patients with primary hypertension and support the hypothesis that APC-R and FV Q506 mutation (factor V Leiden) may be risk factors for arterial thrombosis.

REFERENCES


