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Low Prevalence of Activated Protein C Resistance and Coagulation Factor V Arg^{506} to Gln Mutation among Korean Patients with Deep Vein Thrombosis

Activated protein C (APC) is a naturally occurring anticoagulant that interacts with factor V and VIII to inhibit the clotting cascade. The prevalence of APC resistance among Korean patients with deep vein thrombosis is ill defined. The aim of the present study was to investigate the prevalence of APC resistance and factor V Leiden mutation in Korean patients with deep vein thrombosis. The presence of factor V Leiden mutation was determined in 49 patients who visited Asan Medical Center. APC ratio was performed in 33 individuals from the above 49 patients. Three patients were excluded from the analysis because their baseline aPTT was prolonged. Resistance to APC was diagnosed when the APC ratio was below 2.55. APC resistance was documented in 8 individuals, representing 27% (8/30) of the patients on whom APC resistance test was performed. The 2 patients, who showed APC resistance, were positive for lupus anticoagulant. None of the 49 patients demonstrated factor V Leiden mutation. These findings indicate that factor V Leiden mutation is rare and APC resistance is less prevalent in Korean patients with deep vein thrombosis than in Caucasians. APC resistance not caused by factor V Leiden mutation may be a risk factor for deep vein thrombosis in this population.

Key Words: *Venous thrombosis; Protein C; Factor V; Mutation; Korea*

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INTRODUCTION

Venous thromboembolism is a serious medical problem which causes considerable suffering and occasional death. A number of acquired or genetic risk factors for thrombosis have been described. Genetic risk factors are deficiencies of antithrombin III, protein C, and protein S, as well as dysfibrinogenemia, though together they accounted for only 5% to 10% of cases (1-3). Recently, however, inherited resistance to anticoagulant action of activated protein C (APC) was found to be a factor involved in thrombophilia (4). Resistance to APC has been documented in up to 40% of Caucasian patients with deep vein thrombosis, depending on criteria for patient population selection (5-8). Approximately 90-95% of these cases with inherited APC resistance were caused by a point mutation in factor V gene (factor V Leiden mutation), which will eliminate one of the APC cleavage sites in factor V by replacing it with Arg^{506} by Gln (9-11). However, these studies were performed mostly on Caucasians and there is accumulating evidence for a lower prevalence of APC resistance among populations other than Caucasians (12-15). In Japanese studies, up to 9-18% of thrombosis patients were reported to have APC resistance not associated with factor V Leiden mutation (12, 14, 16). The present study was undertaken to investigate the prevalence of APC resistance and factor V Leiden mutation in Korean patients with deep vein thrombosis.

MATERIALS AND METHODS

Patients

The study population comprised 79 consecutive patients with deep vein thrombosis who visited Asan Medical Center over a 12-month period between March 1996 and February 1997. Thirty patients who did not get blood sampling for factor V Leiden mutation were excluded from the study. APC resistance test was performed in only 33 patients from the above 49 patients.

Table 1. Sites of deep vein thrombosis

Site of thrombosis	No. of patients (N=49)*
Legs	38
Inferior vena cava	3
Intracranial vein	2
Others	6

*Eight patients showed combined pulmonary embolism.

Eleven patients were receiving heparin or warfarin therapy during the investigation. Three of them were excluded from the analysis because their baseline activated partial thromboplastin time (aPTT) was prolonged. Deep vein thrombosis was identified by radionucleide venography with ^{99m}Tc, plain venography, impedance plethysmograpy with duplex scan, or ultrasonography according to standard methods, and pulmonary embolus by ventilation-perfusion lung scan. None of them were wearing an oral contraceptive or showed evidence of pregnancy.

The median age of the patients at the time of study was 42 years (range, 19 to 89). The presenting events were deep vein thrombosis in the leg (38 patients), inferior vena cava (3 patients), and cerebral vessels (2 patients) (Table 1). Eight cases were associated with pulmonary embolism. Factors predisposing the patients to thrombosis were identified in 35% of the patients, the most common factors being trauma (10%) and anti-phospholipid syndrome (8%).

APC resistance assay

For the APC resistance test, patient samples of venous blood were drawn into tubes containing 3.8% sodium citrate and kept on ice. Samples were centrifuged twice at 3,000 rpm for 10 minutes at 4℃ and the aliquots of the platelet-poor plasma stored at -20℃ until use. At the time of testing, specimens were rapidly thawed at 37℃. Activated protein C resistance was assessed using a commercially available kit (Coatest APC, Chromogenix AB, Sweden) according to the manufacturer's instructions. The assay is based on the prolongation of aPTT after the addition of APC. Briefly, 50 μL of thawed plasma was incubated with 50 μL of aPTT-reagent at 37℃ for 5 min, followed by adding 50 μL of either $CaCl_2$ or a mixture of $CaCl_2$ and APC. APC ratio was expressed as a ratio of clotting time in the presence of APC divided by the clotting time in the absence of APC using ACL 300 (Instrumentation Laboratory, Milan, Italy). Normal value for APC resistance was determined by testing samples from 40 healthy volunteers (stored in aliquots at -70℃). The mean APC ratio for healthy subjects was 3.52 [standard deviation (SD) 0.49]. So, APC resistance was diagnosed when the APC ratio was below 2.55 (mean−2SD).

Detection of factor V Leiden mutation

The point mutation in factor V gene was investigated using COASET® FV-506 (Chromogenix AB, Sweden). Genomic DNA was extracted from patient leukocytes collected in EDTA tubes. The region in exon 10, that encodes an APC cleavage site in factor V, was PCR amplified from genomic DNA using the 2 primers: 5'GGA ACA ACA CCA TGA TCA GAG CA3' (forward primer) and 5'TAG CCA GGA GAC CTA ACA TGT TC3' (reverse primer). The 50 μL of amplification mixture contained 10 mM Tris-HCL, pH 8.3, 50 mM KCL, 2.5 mM $MgCl_2$, 200 μM dNTP, 1.25 U Taq polymerase and 25 pmol of primer. The PCR conditions were: 3 minutes initial denaturation at 94℃ followed by 30 cycles of 30 seconds of denaturation at 94℃, 20 seconds of annealing at 62℃, and 30 seconds of extension at 72℃. A final elongation step at 72℃ for 5 minutes was used after the cycling reactions. The 287 bp amplified product was subjected to *Mnl*I digestion and resulting fragments separated by electrophoresis on 2% agarose gel. Three fragments of 157, 93, 37 bp were expected from the 287 bp PCR product from a normal factor V allele. A G to A mutation (nucleotide position 1691) in the codon for Arg^{506} resulted in the loss of one cleavage site and produced fragments of 157 and 130 bp in homozygous controls, and bands of 157, 130, 93, and 37 bp in heterozygous controls.

RESULTS

Prevalence of APC resistance

APC ratio was analyzed in 30 patients. Of this population, 8 patients displayed APC ratio <2.55, representing 27% (8/30) of the patients on whom the APC resistance test was performed. The results of APC ratio in these 8 patients were 1.10, 1.20, 1.40, 1.60, 1.80, 2.00, 2.30, and 2.50, respectively. Two patients who showed APC resistance were positive for lupus anticoagulant. Two patients who received warfarin before the test showed APC resistance.

Prevalence of factor V Leiden mutation

Forty-nine patients were tested for mutation in the factor V gene at codon 506. Factor V Leiden mutation was not seen in any of them including the 8 patients with low APC ratio.

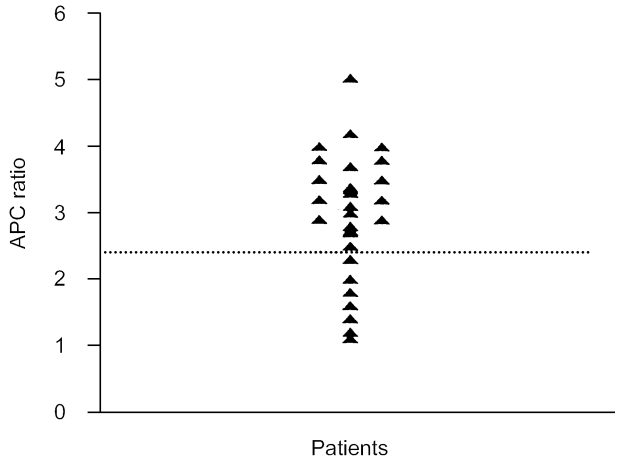

Fig. 1. Results of APC resistance assay in 33 patients investigated for APC resistance. Three patients who showed prolonged baseline aPTT are not included in this figure. An APC ratio <2.55 was considered to be abnormal (indicated by the dashed line).

DISCUSSION

APC resistance is now known to be the most common cause of venous thrombosis, and 40 to 60% of hereditary venous thrombosis in western countries is due to APC resistance. It is also well known that most of the resistance occurs due to a point mutation in factor V molecule (factor V Leiden). However, it appears that factor V Leiden mutation and APC resistance are extremely rare in Asian countries (12-15), and there are no reports of factor V Leiden mutation in Koreans, to date (17-20). Only two cases of APC resistance were reported out of 19 Korean thrombotic patients (21). But factor V Leiden mutation was not checked in these two patients. Our study, on the contrary, showed that 8 out of 30 venous thrombosis patients had abnormally low APC ratios and none of them had factor V Leiden mutation. Higher prevalence of APC resistance in the present study could be due to a higher cutoff value of 2.55 that was the value of mean−2SD. But average APC ratio varies according to the coagulation instruments used (22). In this study, we used ACL 300, which showed higher average APC ratios than other coagulation instruments, such as KC4/10/40, ST-4/ST-888/STA, or Schnitger-Gross. But to increase the specificity, the cutoff value of 2.0 was used in some studies.

APC resistance not associated with factor V Leiden mutation was reported by others also. Bokarewa et al. reported 52 patients with APC resistance, among whom 19 cases had no factor V Leiden mutation, nor could they detect any mutation of other cleavage sites of factor V and factor VIII (23). Similar findings were reported in Japan as well (12, 14, 16). These reports showed 9 to 18% of venous thrombosis in Japanese patients was due to APC resistance without factor V Leiden mutation, quite similar to our study.

The causes of APC resistance without factor V Leiden mutation have just recently begun to be revealed. These causes are lupus anticoagulant and other coagulation factor deficiencies (24, 25), pregnancy (26) and elevated factor VIII level (27), oral contraceptives, and acute thrombotic events (28). We eliminated patients with prolonged aPTT from this study and factor VIII assays were not done. Two of our 8 patients had lupus anticoagulant but none of them were pregnant.

Though cleavage at Arg506 of factor V is the first and essential step for the exposure of subsequent cleavage sites to activated protein C (29), theoretically, it is possible that mutations at other sites of factor V, such as Arg306 and Arg679, as well as mutations of other target molecule, factor VIII, at Arg336, Arg562, and Arg740 could also occur. Roelse et al. (30) and Bokarewa et al. (23) searched for this possibility without success. However, two new mutations of factor V at Arg306 are now reported to be associated with venous thrombosis. One is factor V Cambridge, mutation of Arg306 to Thr, reported by Williamson et al. and is associated with APC resistance (31). The other is Arg306 to Gly, detected in Hong Kong Chinese by Chan et al. (32). They found 3 patients with this mutation (2 with thrombosis). However, APC resistance was checked only in one patient with negative result.

The N-linked carbohydrate moiety on the heavy chain of factor Va is also reported to be responsible for variation in susceptibility to inactivation by APC (33).

Though much more work needs to be done to explain the nature of APC resistance not associated with factor V Leiden, it appears that 5 to 20% (27% in our study) of venous thrombosis patients do have this abnormality.

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