Activated Protein C Resistance, Factor V Leiden, and Central Retinal Vein Occlusion in Young Adults

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**Objective:** To confirm the relationship between resistance to activated protein C (APC), factor V Leiden, and central retinal vein occlusion in young adults as reported in a recent study of patients younger than 50 years.

**Patients and Methods:** Patients younger than 50 years with central retinal vein occlusion were identified from the medical records of the Wills Eye Hospital Retina and Retina Vascular Services. Blood samples were taken from each patient and analyzed for resistance to APC and identification of factor V Leiden.

**Results:** Only 1 (4.7%) of 21 patients evidenced resistance to APC and the presence of factor V Leiden. This patient was also the only one to report a family history of thrombotic disease.

**Conclusions:** We were unable to confirm the high percentage of resistance to APC among young adult patients with central retinal vein occlusion. The finding of resistance to APC in only 1 (4.7%) of 21 patients is similar to that found in the general population.


Central retinal vein occlusion (CRVO) is usually found among older patients and is usually associated with systemic vascular disease. Central retinal vein occlusion in younger patients is an uncommon finding; 7.5% to 19.8% of CRVOs have been reported to occur in patients younger than 50 years, but these rates may be overestimated as the younger adult who develops a CRVO is more likely to be referred to a retinal specialist because of the rarity of the condition. Associated medical conditions in the young adult who experiences a CRVO may include hypertension, hyperlipidemia, migraine, carotid artery disease, or hypercoagulability. However, identifiable associated medical disease is less common than that found in the older adult population in whom CRVO develops.

Genetic defects known to predispose individuals to venous thrombosis include deficiencies of antithrombin III, protein C, and protein S. In 1992, hereditary activated protein C (APC) resistance was identified as a basis for a majority of cases of familial thrombosis. The disorder responsible for this resistance in more than 90% of cases was shown to be a single point mutation in coagulation factor V gene at nucleotide position 1691 coding for a mutant factor V rendering it resistant to inactivation by protein C. This mutant factor V is commonly referred to as factor V Leiden. Activated protein C is involved in the delicate balance of procoagulant and anticoagulant forces of the coagulation cascade. Activated protein C inhibits coagulation through the inactivation of membrane-bound activated factor V and activated factor VIII. Activated protein C resistance results in hypercoagulability and an increased risk of deep vein thrombosis.

While resistance to APC is most commonly caused by the presence of factor V Leiden, the results of the clotting test for resistance to APC can be positive without factor V Leiden. Causes of acquired resistance to APC include pregnancy, the use of oral contraceptives, and the presence of lupus anticoagulant. To confirm the presence of the mutant factor V Leiden, genetic analysis may be performed to directly assay for the genetic abnormality.

Resistance to APC has been described in patients with idiopathic CRVO and in those with bilateral and recurrent CRVOs. In a recent study of 31 young adults with CRVO, 26% of patients younger than 50 years and 36% of patients younger than 45 years evidenced resistance to APC, identifying APC resis-
Patients and Methods

Patients

Patients were selected from a search of the patient records of the Retina Service and Retina Vascular Service of the Wills Eye Hospital, Philadelphia, Pa, as well as recruitment of patients on a prospective basis during a 1-year period. A computer search of the patient records of the subspecialty group practice of one of us (W.E.B.) as well as of the patient records of the Retina Vascular Service was performed identifying patients 50 years old or younger who were coded as having “central retinal vein occlusion.” The records reflect approximately 10 years of patient visits. Each patient record was screened to assure correct diagnosis coding, and eligible patients were contacted by telephone to invite participation in the study. As resistance to APC usually reflects a genetic abnormality, it was possible to recruit patients who were not within the acute stages of CRVO. We identified 39 patients who met the eligibility requirements and 21 were successfully contacted by telephone. Eleven patients agreed to participate. The remaining patients did not wish to travel to the city hospital laboratory to have the necessary blood work performed. Ten additional patients were identified prospectively and each agreed to participate. In total, 21 patients agreed to participate in the study, and blood samples were obtained from each of the patients after informed consent was obtained.

Fifteen men (71%) and 6 women (29%) participated in the study. The average age of the patients at the time of the diagnosis of CRVO was 42.1 years old. No patient was older than 50 at the time he or she had the CRVO. Only 1 patient related a family history of venous occlusive disease. Four patients were African American and the remaining 17 patients were white Americans. One patient had a history of a spontaneous abortion and positive antibodies to the antiphospholipid antigen on initial testing, but repeated testing for lupus anticoagulant did not confirm the presence of the antibodies. Two patients had positive results from antinuclear antibody testing, but rheumatologic evaluation did not reveal specific disease in either patient. In all of the remaining patients, results of laboratory testing for protein C, protein S, antithrombin III, anticalciolipin antibody, complete blood cell count, erythrocyte sedimentation rate, activated partial thromboplastin time, and prothrombin time, where tested, were normal.

Methods

Laboratory testing for resistance to APC and factor V Leiden was performed in the Special Hemostasis Laboratory of the Cardeza Foundation for Hematologic Research of the Thomas Jefferson University in Philadelphia. Activated protein C resistance was determined using an APC resistance kit (Coatest-APC Resistance V kit, Chromogenix, Molndal, Sweden) according to the manufacturer’s directions. This test provides higher than 95% sensitivity and specificity for factor V Leiden mutation. Factor V Leiden mutation was identified by detection of a change in the MnI restriction enzyme digestion pattern of the patient’s genomic DNA. DNA was extracted from 5 µL of anticoagulated (0.38% sodium citrate) whole blood using the Genereleaser (Bioventures, Inc, Murfreesboro, Tenn) according to the manufacturer’s directions. DNA was amplified by polymerase chain reaction followed by restriction enzyme digestion with HpaII as described by Koelleman et al. Samples from patients known to be positive and negative for factor V Leiden mutation, as well as a control sample without DNA, were run with each patient sample. Aliquots of the polymerase chain reactions were separated by gel electrophoresis in ethidium bromide containing 3% agarose gel. The gels were photographed under UV illumination and the DNA restriction patterns compared.

Results

Resistance to APC was seen in only 1 (4.7%) of the 21 patients studied (95% confidence interval = 0.572, 1.428, Poisson distribution.) The average APC ratio of the normal patients was 2.9 (range, 2.3 to > 4.0). The APC ratio of the one patient who evidenced resistance to APC was 1.8. This patient also evidenced factor V Leiden. His father had a history of deep vein thrombosis and prolonged activated partial thromboplastin time. This family member had not had his condition evaluated for genetic abnormalities of coagulation. No patient evidenced presence of factor V Leiden with negative test findings for resistance to APC.

Of the 20 patients with no resistance to APC, 14 evidenced a nonischemic CRVO. The one patient with resistance to APC suffered an ischemic CRVO.

Comment

Among the 21 young adult patients with CRVO included in our study, only 1 (4.7%) showed resistance to APC and the presence of factor V Leiden mutation. This stands in contrast to the study of Larsson and colleagues in which 8 patients (26%) younger than 50 years and 11 patients (36%) younger than 45 years were resistant to APC. The prevalence of resistance to APC among the general population is approximately 5% in European population studies, and among white Americans the carrier frequency of factor V Leiden is 5.27%. From our study, it does not appear that there is a higher prevalence of resistance to APC among young patients with CRVO than there is in the general population. Similarly, in a study of genetically affected family members in a study of 50 APC-resistant families, only 1 case of CRVO was found. Among those patients in our study evaluated for other inherited disorders of coagulation predisposing to thrombosis, no patient evidenced protein C, protein S, or antithrombin III deficiencies. As in the study of Larsson and colleagues, the patients included in this study were chosen from the pa-
tient records of a university-associated ophthalmology hospital. Resistance to APC usually results from an inherited genetic abnormality. In our study, we were testing specifically for the inherited mutation, factor V Leiden. Therefore, the follow-up interval or timing of the testing relative to the occurrence of the CRVO is not important. The median age of 43.5 years (average age, 42 years) is similar to the median age of 40 years in the study of Larsson et al. One significant difference was the presence of 5 patients in their study with a family history of thrombosis. Interestingly, the only patient who related a family history of thrombosis in the present study also evidenced resistance to APC. This may reflect a selection bias affecting the differences in the results.

Other potential sources of bias include the means of selection of patients for this study. The patients recruited included both urban and suburban populations. The patients recruited retrospectively came from both the general clinic population of Wills Eye Hospital (the Retina Vascular Service) and the referral patients of the private practice of members of the Retina Service of Wills Eye Hospital. Six of 11 patients recruited retrospectively were from the general service and 5 from the private practice. Patients agreeing to participate generally represent the overall population of the greater Philadelphia metropolitan area. No attempt was made to specifically recruit patients with a family history of thrombophilia or recurrent thrombosis.

Resistance to APC that was not the result of factor V Leiden mutation has been reported in patients with acute strokes and in pregnancy. It is not clear that in these settings the APC resistance results in an increased thrombotic risk. The assay used by Larsson et al. in their study of patients with CRVO would also detect these presumed acquired states of APC resistance. The assay method that we used is highly sensitive and specific for factor V Leiden mutation. It is possible that the difference between our results and those of Larsson et al is due to the use of different assays. However, we did use an assay similar to that used by Larsson et al in 6 patients and we obtained results identical to that obtained with the more specific assay.

Factor V Leiden mutation is highly prevalent in the general population. As an inherited genetic disorder, it causes a life-long increased risk of thrombosis and is inherited as an autosomal dominant trait. In extended family studies, approximately 25% of relatives with APC resistance had suffered a thrombotic event by the age of 50. The presence of APC resistance, when combined with other risk factors for venous thrombosis such as pregnancy, cigarette smoking, oral contraceptive use, surgery, or other genetic anticoagulant defects, afflicts the patient with a considerable risk for venous thrombosis. In the young patient who presents with a CRVO, especially if there is a family history of thrombotic disease or concomitant risk factors for thrombotic disease, screening for resistance to APC is indicated, and when results of such tests are positive, the patient should undergo genetic analysis to confirm the presence of factor V Leiden. Such patients may be counseled regarding the avoidance of risk factors that may increase their susceptibility to thrombotic disease, including the use of tobacco or oral contraceptives. These young patients may also benefit from prophylaxis against thrombosis if surgery or immobilization is required later in life. This present study, however, does not confirm that there is a 4 to 5 time greater risk for resistance to APC among young patients with CRVO than there is among the normal population.

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REFERENCES