SHORT COMMUNICATION

FACTOR V LEIDEN IN YOUNG PATIENTS WITH THROMBOPHILIA

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Venous thrombosis presents major health problem worldwide. About 10% of hospital deaths are because of deep vein thrombosis (DVT) with consequent pulmonary embolism (PE), whereas half of them are patients who otherwise have a non-fatal disease.1 The risk of developing venous thrombosis, particularly at younger age (<45 years) is increased from 5 to 80-fold in the presence of certain genetic abnormalities (depending upon the type of abnormality, zygosity and multiplicity of involved factors).2 Of these by far the commonest reported genetic abnormality is of factor V (factor V Leiden) that occurs in 20-60% of patients with venous thrombosis.

Factor V Leiden (FVL) results from a point mutation causing a single nucleotide change at position 506.3 This change makes the activated factor V (Va) resistant to cleavage by activated protein C (APC) whereas the procoagulant activity of Va remains undeterred thus resulting in thrombophilia.4 Environmental factors then interact with this inherited abnormality to increase the risk of thrombosis several times.5

This study addresses the frequency of FVL in Pakistani patients with thrombophilia.

All patients with the occurrence of a venous or arterial thrombo-embolic event at an age of <45 years, reporting to any of the hospitals in Rawalpindi-Islamabad, during the period of study i.e. 1st January 1999 till 31st December 1999, were included. Patients with a previously confirmed abnormality in coagulation/fibrinolytic pathway, oral anticoagulants intake for >6 months or suffering from any malignancy were excluded from the study.

Eight ml of venous blood was collected from each patient, of which 1.8 ml was mixed with 0.2 ml tri-sodium citrate (100 mmol/l) for use in clotting tests. Remaining was equally divided in two bottles containing dipotassium EDTA and was used for blood counts and extraction of DNA.

DNA was extracted by standard phenol-chloroform extraction method and was frozen at -20°C until analyzed. Citrated blood was centrifuged at 2000g for 20 minutes to obtain platelet poor plasma. One portion of this plasma was used to carry out prothrombin time (PT) and partial thromboplastin time with kaolin (PTTK) and other portion was frozen at -70°C until screening test for activated protein C (APC) system was performed using standard manufacturer’s instructions.

APC system was tested using ProC-Global assay (Dade Behring, Marburg, Germany). The results were considered valid only if the values of QC plasma were within the confidence range. A decreased response, normalized ratio (NR) below 0.80, is suggestive of presence of homozygous FVL mutation.

FVL mutation was detected by restricted fragment length polymorphism (RFLP) for a 220 base pair (bp) (exon 10-intron 10) fragment of factor V. The fragment was amplified by polymerase chain reaction (PCR) using the following pair of primers:

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5' \rightarrow \text{TGC, CCA, GTG, CTT, AAC, AAG, ACC, AAC} \\
3' \rightarrow \text{CTT, GAA, GGA, AAT, GCC, CCA, TTA}
\]

Amplification involved 36 cycles consisting of denaturation at 94°C for 40 seconds, annealing at 55°C for 40 seconds and extension at 71°C for 2 minutes in the presence of 2 units of Taq polymerase. Subsequently 220 bp fragment was digested by 0.4 units of Mn/1 at 37°C for 16 hours. Mn/1 digests normal factor V allele into three fragments of 37, 67, 116 bp each whereas FVL allele is cleaved only in two fragments of 67 and 153 bp each. Finally, the digestion products were separated by electrophoresis on 6% acrylamide gel and stained with silver nitrate.

A total of 46 patients with established thromboembolic disease were recruited in the study. The diagnosis was based on history, clinical findings and investigations e.g., Doppler studies, CT scan, lung perfusion scan and venography.

Patient population included 36 (78.3%) males and 10 (21.7%) females. Age ranged from 12 to 45 years with a median of 30 years. Based on the clinical spectrum patients were divided into three groups.

Group-I had DVT/pulmonary embolism (n=20), group-II had stroke (n=16) and group-III were females with recurrent fetal loss (n=10).

No difference in the clinical and laboratory parameters was observed between the three groups.

Of these, 11 patients were already on oral anticoagulants (Warfarin). Comparison of clinical and laboratory parameters of non-anticoagulated and anticoagulated patients are shown in Table I.

Table I: Comparison of non-anticoagulated and anticoagulated patients are shown in Table I.

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NR with ProC Global test was abnormal (<0.80) only in patients on oral anticoagulants. All other patients had NR
Of these, only 2 were found to be heterozygotes for FVL by PCR. No homozygote was detected.

The results had a sensitivity of 100% and specificity of 76%. Negative predictive value was 100% whereas positive predictive value was only 18%.

In recent past resistance to APC caused by R506Q mutation in factor V gene (factor V Leiden) has come up as the most important single cause of thrombophilia having been detected in 10-60% cases of thromboembolism. Its incidence from India is only 3%. In this study only 2 (4.3%) of 46 patients studied were found to be carrying this mutation in heterozygous state.

Screening for APC resistance was done using ProC Global. NR was used as the diagnostic parameter, which is though less specific, is more sensitive. Normal NR stated by the manufacturers is 0.94 (CI 0.69-1.56) for PCAT and 0.99 (CI 0.86-1.10) for PCAT/FV. We used a safe cut off point of 0.8 for PCAT. In our study all positive patients were on Warfarin therapy. The NR should have corrected on addition of factor V deficient plasma in all patients because this fact alone constitutes the screening parameter for FVL. In our study partial correction was observed only in one patient, in others there was either no correction or NR was prolonged. There are several possible explanations for this. Manufacturers state that contamination of frozen specimen with cellular debris may result in this. Inactivation of protein C during preparation of factor V deficient plasma may also cause the same problem. Reduction in activity of other factors in preparation is ruled out by the fact that none of the PCAT/FV was less than 120 seconds.

This test has 100% sensitivity to the presence of FVL. However, it also carries 90% sensitivity to protein C levels of <70% of normal and protein S levels of <30%. Both these proteins are reduced in patients on Warfarin therapy. This may be the reason that it was positive in all patients on Warfarin therapy. Increased levels of factors V and VIII as well as presence of lupus anticoagulant may also result in positive test.

FVL mutation was only demonstrated by PCR in 2 of these 11(18%) patients with an overall incidence of 4.3% (2/46). Thus, it is clear that molecular technique is more specific and sensitive to demonstrate FVL mutation but ProC Global is a useful test for selecting patients in whom this procedure is to be carried out.

REFERENCES