Role of Thrombophilic Mutations in Thrombosis

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Background

The present study looked at 9 mutations (mentioned in the findings) in young Indian thrombophiliacs for the first time to extensively study their role in the pathogenesis of thrombosis.

Aims & Objectives

To extensively study the role of thrombophilic mutations in the pathogenesis of thrombosis.

Material & Methods

This study included all cases of thromboembolic disorders in individuals less than 45 years. 15 cases were studied for the mutations while another 15 normal healthy young individuals were studied to act as a control. The patients were worked up for coagulation based markers for thrombophilia which are routinely available. For the mutation analysis,

- The Single Tube Multiplex PCR system was designed to identify the mutations and their polymorphism.
- The blood sample was collected in EDTA vacutainers.
- The DNA was extracted by the column method with the reagents provided in the kit.
- The DNA was mixed with the forward and reverse primers for mutations, buffer and dNTPs
- PCR was carried out in thermal cycler as per protocol.
- The particular mutation or wild product was amplified as per the patient's genotype.
- The PCR products were biotinylated in the tubes and incubated. The biotinylated product were then reverse dot blotted on to the strip with prefixed oligonucleotides which gave a positive colour band as per the mutation or normal gene amplified.
- Detection of the specifically bound mutant or wild type was visible by enzyme colour reaction which was interpreted as 3 patterns.
- These were- wild homozygous (normal pattern with only one band), both wild and mutant pattern (heterozygous for mutation) and only mutant positive as mutant homozygous.
- Thus for nine mutations, a total of 27 polymorphisms were studied.
- These polymorphisms were correlated to the clinical features of the patient.
- The clinical presentation, age, family history, presence of multiple mutations, interaction with environmental factors and any correlation if any, were noted in a performa.
- Analysis of the data was done by using appropriate statistical tests.
- The study was done for finding out the proportion of the various genetic mutations {Factor V Leiden (G1691A; R506Q) : FV R2 haplotype (H1299R): Prothrombin (PTH & Factor II): G20210A MTHFR C667T: MTHFR A1298C Factor XIII (FXIII) V34L: PAI – 1,Serpin E1 4G/5G: EPCR 4600 A>G (A3 haplotype EPCR 4678 G>C (A1 haplotype) } and their polymorphism in Indian patients < 45 yrs of age presenting with thromboembolic disorders.
- The various mutations and their polymorphisms with the clinical presentations of thrombotic disorders in young Indians were then correlated.
- The statistical data and findings of the project will be discussed during the conference.

Results & Conclusions

The study has helped in providing data on the prevalence of nine mutations encountered in thrombophiliac patients and the role of these in the pathogenesis of thrombosis. Thus the preventive strategies for this disease which causes morbidity, mortality and loss of manhours in our young soldiers is now possible.