The THAI Journal of SURGERY 2006; 27:11-14.
Official Publication of the Royal College of Surgeons of Thailand

Factor V Leiden and Prothrombin Gene Mutation in Deep Vein Thrombosis

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Abstract ***Objectives:*** **To study the prevalence of genetic risk variation in factor V gene (G1691A Leiden mutation) and prothrombin gene (G20210A)**

Materials and Methods: **Polymerase chain reaction techniques were performed in 50 healthy Thais and 48 patients with deep vein thrombosis.**

Results: **The prevalence of both factor V Leiden and prothrombin G20210A gene mutation in a control group was zero. Of 48 patients with DVT, the disease was located in the lower extremities, none was a carrier of factor V Leiden or the prothrombin gene mutation. Our finding confirms the previous study by Angchaisuksiri et al who found a very low prevalence of this mutation in Thai patients with DVT.**

Conclusion: **The screening for factor V Leiden and Prothrombin gene mutation is not beneficial and may not be cost-effective in Thai patients with DVT.**

Key words: **factor V Leiden; prothrombin G20210A gene mutation**

Introduction

Venous thrombosis is a serious health problem causing significant morbidity and mortality. It may be fatal by its complication of pulmonary embolism. Fatality rate of venous thrombosis is estimated at 1% to 2%. Deep vein thrombosis (DVT) is common in Caucasians with the incidence of 1 per 1000 individuals per year.[1] It has been reported that DVT is less common among Thais than Caucasians.[2]

The pathogenesis of venous thrombosis is complex, involving the interaction of acquired risk factors with several genetic predispositions. Acquired risk factors including pregnancy, oral contraceptive use, estrogen therapy, obesity, malignancy, diabetic mellitus, venous stasis from immobility, trauma and post-operation can precipitate thrombosis. Genetic risk factors for thrombosis include the abnormalities of protein C, protein S and Antithrombin III which decrease concentration or function leading to a considerably increased risk (approximately 10-fold) for venous thrombosis.[3]

In 1993, Activated protein C resistance that was subsequently linked to a single base-pair mutation in factor V gene, known as the factor V Leiden mutation, has been described.[4] Several studies demonstrated that factor V Leiden is the most frequent hereditary cause of venous thrombosis in Caucasian[3] and the second most frequent genetic cause is prothrombin G20210A mutation.[5] Both of these genetic abnormalities are commonly found in patients with DVT, with prevalence varying between 20% and 50% for factor V Leiden and between 5% and 19% for prothrombin gene mutation.[6] Previous research

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indicated that the presence of factor V mutation increases risk for venous thrombosis 7-fold in heterozygotes and 80-fold in homozygotes.[7] The incidence of venous thrombosis in homozygotes is almost 100% and 10% in heterozygotes.

Factor V Leiden is the result of the replacement of an arginine at position 506 (coding triplet CGA) with a glutamine (coding triplet CAA) by substitution of G to A at nucleotide position 1691. Thus, factor V Leiden leads to the phenomenon of resistance to the anticoagulant activity of APC. There is a significant risk for thrombosis. The risk is higher when clinical or environmental risk conditions are also present. The high prevalence in Europeans suggests that screening for this mutation should be considered in these circumstances.

Prothrombin gene mutation is associated with a 2.1-fold increased risk of venous thrombosis that concerns a G to A transition in nucleotide 20210 in the 3-untranslated region of the prothrombin gene. This mutation interacts strongly with some other genetic risk factors such as factor V Leiden and protein S deficiency and hence significantly increases risk of venous thrombosis.

The different population frequencies and the geographic variation have implications for the relevance of the mutation and the occurrence of thrombosis in various populations. Previous results of the studies of the prevalence of factor V Leiden and prothrombin gene mutation in Thai patients with DVT were controversial. Prayoonwiwat W et al. reported that factor V Leiden mutation was related to venous thrombosis in 11%[8], whereas Angchaisuksiri P et al. found a very low prevalence of this mutation in DVT in Thai patients.[9]

The purpose of this study was to investigate the prevalence of these mutations which was important in the evaluation of genetic risk and prediction of outcome of deep vein thrombosis in Thai populations.

Materials and Methods

The data of 48 Thai patients (23 males and 25 females) with clinical features and findings of deep vein thrombosis confirmed by Duplex Doppler ultrasonography, at Siriraj Hospital, Faculty of Medicine, Mahidol University, were collected between July 2001 and 2002.

Clinical data including age, sex, personal and family history for thromboembolic evidences, known risk factors for venous thromboembolism including surgical procedure and major trauma in the previous 3 months, child birth, pregnancy, oral contraceptives use, hormone replacement therapy, malignancy, lengthy air travel (over 6 hours) and immobilization were collected.[10] The diagnosis of DVT of the limb was objectively made by Duplex Doppler ultrasound. We investigated 50 unselected apparently healthy Thai subjects without history of venous thromboembolism as control at the same time. All subjects were fully informed of the aims of the survey and gave their consents. Venous bloods were collected on the same day from all individuals in EDTA for DNA analysis.

Molecular analysis was performed after polymerase chain reaction (PCR) amplification of genomic DNA, restriction enzymes digestion, and agarose gel electrophoresis, using primer of factor V Leiden (5' TGTTATCACACTGCTGCTTAA3'), prothrombin gene mutation (5'TCTAGAAACAGTTGCCTGGC3') and restriction enzymes as described for factor V Leiden and prothrombin G20210A mutation.

Factor V Leiden G1691A (174 bp) was identified by using PCR followed by HindIII digestion. The procedure was adapted from that described by He'zard N, et al. in 1997.[11] The identification of the 20210A allele of the prothrombin gene (340 bp) was carried out according to the method described by Poort et al.[12] Factor IX was an internal control (250 bp) with forward primer: 5'CTCCTGCAGCATTGAGGGAGATGGACA TT3', following the procedure by He'zard N, et al.[10]

For isolated genomic DNA, Protein K and phenol/chloroform were used in extraction protocol for genomic DNA from venous (EDTA) blood.

The point mutation in Factor V Leiden (G1691A) and Factor II or prothrombin (G20210A) were detected by multiplex-allele specific amplification (ASA)

PCR amplification

Polymerase chain reaction (PCR) was performed by cycling samples containing template DNA mixed with sequence-specific oligonucleotide primers through three temperature incubation in the presence of Thermus aquaticus (Taq) DNA polymerase. Each PCR reaction consists of 50 ng of genomic DNA, 1.25 µl of FV Leiden primers set, 0.5 µl of PT primers set, 0.1 µl of 5U/µl TaqDNA polymerase, 0.625 µl of

10 mM dNTPmix, 2.5 µl of 10X PCR buffer (160 mM $(NH_4)_2SO_4$, 1M Tris-HCL pH 8.3, 0.1% Tween -20) and sterile-distilled water added to a total volume of 25 µl. PCR cycles for both wild type and mutant reaction of amplification were performed and consisted of 30 second denaturation at 94˚C, 30 second annealing temperature and one-half minutes extention at 72˚C after an initial 7 minutes denaturation step at 95˚C. A final extension for 10 minutes at 72˚C was also performed.

Agarose gel electrophoresis

Agarose gel electrophoresis was performed in a horizontal submerged gel apparatus. The 2.5% of LE agarose gel was prepared as follows. Electrophoresis buffer (0.5 × TBE) was prepared from stock solution (5 ×TBE). Buffer was added and melt in the microwave oven. On cooling to below 60-70˚C, ethidium bromide (1 µg/ml) was added for 15 min. Loading buffer was added to the PCR products which were transferred into the wells, and electrophoresis was performed at constant voltage (130 volts) for 50 min. The gel was transilluminated with short wave ultraviolet light and the DNA was visualized by photography using a UV camera. The gel was then taken into documentation analysis machine (syngene) for a photo.

Results

Deep vein thrombosis was confirmed by Duplex Doppler ultrasonography in 48 patients. In all cases of DVT, the disease was located in the lower extremities and affected the proximal segments of the veins (popliteal, femoral, iliac veins) and none were related with pulmonary embolism. The median age at the time of thrombosis was 38.2 (ranged 25-72) years.

Based on the patients' data, associated acquired risks included smoking in 8/48, diabetes in 4/48, hypertension in 6/48 and heart disease in 1/48. There was no associated pregnancy, immobilization, malignancy, post-operative state, or the use of oral contraceptive.

We also identified 50 healthy subjects as control and none were carriers of factor V Leiden or G20210A genotype. The prevalence of factor V Leiden and the prothrombin G20210A gene mutation in a group of 48 patients were zero percent.

This study indicates that Thai patients with or without DVT are not associated with factor V Leiden and the prothrombin G20210A gene mutation. The study shows that it is not important to identify these genetic factors in Thais with DVT and Thai population as the screening test.

Discussion

The pathogenesis of deep vein thrombosis is complex and involves the interaction of acquired risk factors with several genetic predispositions. DVT appears to be an interesting model of multifactorial disease. Factor V Leiden seems to be the most common mutation which probably has a role in the development of DVT.[13]

Factor V Leiden mutation is rather common in Caucasian populations, but there are important regional differences in prevalence (2% to 16%).[14] Factor V Leiden is of high prevalence in Europeans and the screening for this mutation should be considered in these circumstances.[15] The prevalence in non-Europeans is not known, but venous thrombosis is rare in Southeast Asia. The prevalence of factor V Leiden allele in Asia (indonesia 105, Taiwan 83, Hong Kong 48) was zero percent, both the homozygotes and heterozygotes.[15] It is interesting that factor V Leiden, reaching such high frequencies in Europe, is rare in the rest of the world.

A mutation in the 3'-untranslated region of prothrombin at position 20210 (PT20210A) is associated both with prothrombin levels and the risk of thrombosis.[5] The 20210A allele of the prothrombin gene possibly has a similar distinctive racial and/or geographic distribution as has been described for the FV mutant.[15]

In Thailand, two previous studies[8,9] had different results in the prevalence of factor V Leiden and Prothrombin gene mutation. This study identified no mutation in factor V Leiden and prothrombin gene in both groups of subject. The results of this study were in accordance with previous study by Angchaisuksiri P, et al.[9] that found a very low prevalence of these mutation in Thai patients with DVT. However, our method of study by using PCR followed by *Hin*dIII digestion was similar to Angchaisuksiri P, et al.

Although, factor V Leiden is an important risk factor for venous thrombosis, the prevalence of factor V Leiden was 15.7% in patients with DVT and 14.1% in

patients with PE compared with 5.3% in patients without DVT and/or PE.[16] The prevalence of the prothrombin G20210A mutation did not differ among the groups.[16]

For the family risk of venous thrombosis, there was a slightly lower risk of venous thrombosis in carriers of the prothrombin gene mutation than in carriers of the factor V Leiden.[17] In Thailand, factor V Leiden gene mutation and prothrombin G20210A gene mutation are not associated genetic risk factor in Thai population. Factor V Leiden and factor II mutation are not associated with DVT in Thai patients. The screening for Factor V Leiden and Prothrombin gene mutation is then not beneficial and may not be cost-effective in Thai patients with DVT.

When thrombosis is invariably the result of interaction between genetic and environment risk factors, removal of all genetic risk factors would lead to 100% reduction of the occurrence of thrombosis, as would the removal of all environmental risk factors.[18] Then the confirmation of the prevalence of two genetic factors in Thai population with and without DVT is important.

Conclusion

Venous thrombosis is a multicausal disease. Risk factors resulting from genetics, environment and behavior may bring about thrombosis. Factor V Leiden mutation is rather common in Caucasian populations and is of high prevalence in Europeans. The screening for this mutation should be considered in these circumstances. Our study identified no mutation in factor V Leiden and prothrombin gene in both groups of subject. The results of this study were in accordance with previous study by Angchaisuksiri P, et al.[9] that found a very low prevalence of these mutation in Thai patients with DVT. The evidence of no prevalence of factor V Leiden and prothrombin gene mutation in Thai population suggests that prospective trials to assess the medical and economic desirability of screening test in Thai patients with DVT should not be undertaken.

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