Prevalence of Factor V Leiden in Thai Blood Donors

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Abstract

Factor V Leiden was recently found to be the most common cause of familial venous thrombosis in the European population. We have studied the prevalence of factor V Leiden by DNA analysis among 500 Thai blood donors (male 285, female 215). Their ages ranged from 18 to 60 years with a mean of 33 years and 2 months. All of them were healthy voluntary blood-donors who met the standard criteria of the American Association of Blood Banks. No history of thrombosis was found. The results revealed that factor V Leiden was not present among 1,000 chromosomes from Thai blood donors. This suggests that factor V Leiden is not the common genetic predisposing factor of venous thrombosis in the Thai population as compared to the European population.

Key word : Factor V Leiden, Venous Thrombosis

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Thromboembolism has gradually become a health problem in the Thai population. The pathogenesis is complex, involving the interaction of underlying genetic predisposing factors and acquired risk factors[1] such as obesity, hypertension and smoking. However, the occurrence of the hereditary deficiency of antithrombin III, protein C or protein S involves only 20 per cent of familial thrombosis cases[2]. Recently, the resistance to activated protein C was found to be the most

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common cause of 20-40 per cent of familial venous thrombosis cases[3]. More than 95 per cent of the cases of resistance to activated protein C are caused by a point mutation in the factor V gene (arginine 506 to glutamine) referred to as factor V Leiden[4]. It results from a substitution of adenine (A) for guanine (G) at the nucleotide 1691 of the factor V gene. Heterozygotes for this mutation have a sevenfold increased risk of venous thrombosis while the homozygotes are eighty times more susceptible[4-6]. However, factor V Leiden was not found in any of 272 cases from Asia which included Indonesians, Sumatrans, Taiwan Aborigines, Mongolians, and Hong Kong Chinese [7]. It is worth knowing if the prevalence of factor V Leiden among the Thai population is low. Here, we report the prevalence of factor V Leiden among 500 Thai blood donors.

SUBJECTS AND METHOD

Subjects

Five hundred regular blood donors at the Blood Bank, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Bangkok were enrolled in the study from January 1998 to September 1999. One milliliter of whole blood was mixed with EDTA as an anticoagulant and kept in a 4°C refrigerator.

Additionally, 18 patients from the Department of Pediatrics, Ramathibodi Hospital, whose ages ranged from 4 to 14 years, experiencing thromboembolism, were included in the study. They included nine boys and nine girls. The sites of thrombosis involved the lungs, brain, and, deep veins of the legs. The etiologies of thrombosis were not known except in three patients who had lupus anticoagulant, polycythemia and Moya-Moya disease. Their levels of antithrombin III, protein C and protein S were normal except in one patient with lupus anticoagulant. His protein S level was 0.1 unit/ml while the normal values were 0.7 to 1 unit/ml.

Method

DNA was extracted from whole blood using a 5 per cent suspension of Chelex by the following procedures[8]. First, three µl of whole blood were added to one ml of sterile distilled water, and incubated at room temperature for 15-30 minutes, mixed occasionally by inversion or gentle vortexing. Next, it was centrifuged in a microcentrifuge at 10,000–15,000 rpm for 2-3 minutes and the supernatant was carefully removed and discarded. Then, the 5 per cent suspension of Chelex was added to a final volume of 200 µl and incubated at 56°C for 15-30 minutes. After, it was vortexed at high speed for 2-3 seconds and incubated in a boiling water bath for 8 minutes. Then, it was vortexed at high speed for another 5-10 seconds and spun in a microcentrifuge at 15,000 rpm for 2-3 minutes. As a result, 10 µl of the supernatant was ready for DNA amplification.

Three primers,[9,10] FV3: 5/ -CATGAGA GACATCGCCTCTG-3/, FV6:5/-GACCTAAC ATGTTCTAGCCAGAAG-3/ and FV7:5/-AAGGAC AAAAGTACCTGTATTCCA-3/ were used as follows: 10 µl of genomic DNA was amplified in a 50 µl reaction volume consisting of Cetus buffer (10 mM Tris-HCl pH 8.3, 50 mMKCl, 1.5 mM MgCl 2, 0.01% gelatin), 200 µM dNTPs (Pharmacia), 30 pmole of primers FV3 and FV 6, and 1 unit of Taq Polymerase (Perkin-Elmer). Following an initial denaturation at 95°C for 7 minutes and thermocycling of 94°C for 1 minute, 67°C for 2 minutes and 72°C for 5 minutes were carried out for a total of 35 cycles and a final extension at 72°C for 10 minutes. The amplified product of 147 bp was obtained. Following amplification, 20 µl of products were digested with the restriction enzyme *Mnl* I (Biolabs, New England) and incubated at 37°C overnight according to the manufacturer's instructions. Then, the size-fractionation by gel electrophoresis on 12 per cent native polyacrylamide gel was performed. The homozygotes for the normal allele at 1691 (G/G) generated three bands of 85, 37 and 25 bp. The suspected heterozygotes at 1691 (A/G) gave four bands of 122, 85, 37 and 25 bp while the suspected homozygotes at 1691 (A/A) gave only two bands of 122 and 25 bp.

In cases of suspected heterozygotes or homozygotes at 1691 (G → A), the second more specific nested amplification was performed. One microlitre of the first amplified product was used as a template. The amplification condition was the same as the first amplification except for primers FV 3 and FV 7. The amplified product

of 94 bp was obtained. Following amplification, 20 ml of the product were digested with the restriction enzyme *Nla* III (Biolabs, New England) and incubated at 37°C overnight according to the manufacturer's instructions. Then, the size-fractionation by gel electrophoresis on 12 per cent native polyacrylamide gel was performed. The results of two bands of 71 and 22 bp were found only when A was present at position 1691.

RESULTS

Five hundred regular blood donors (285 males, 215 females) were included. Their ages ranged from 18 to 60 years with a mean of 33 years and 2 months. All of them were healthy voluntary blood donors who met the standard criteria recommended by the American Association of Blood Banks. No history of thrombosis was found.

The amplified product from the first pair of primers, FV 3 and FV 6 was 147 bp. After digesting with the enzyme *Mnl* I, all of the blood donors generated three bands of 85, 37 and 25 bp. Therefore, the results revealed that factor V Leiden was not present among 1,000 chromosomes from Thai blood donors. Also, factor V Leiden was not present among 36 chromosomes from children with thromboembolism.

A known DNA sample from a heterozygote of factor V Leiden was included as a positive control. Her primary amplified product from the first pair of primers, FV 3 and FV 6, was digested with the enzyme *Mnl* I. She generated four bands of 122, 85, 37 and 25 bp. Then, the nested amplified product from the second pair of primers, FV 3 and FV 7, was digested with the enzyme *Nla*III. She possessed three bands of 94, 71 and 22 bp. The results confirmed that she was heterozygous for factor V Leiden.

DISCUSSION

Factor V Leiden has been shown to be the most common cause of familial venous thrombosis in western countries. The reported prevalence in Caucasian populations is 3-7 per cent (5,11). Thromboembolism in the Thai population is actually lower than western countries(12,13). The habitual ingestion of garlic and chile peppers in high fiber and low fat diets are also contributing factors. However, the underlying genetic predisposing factor towards the risk of thromboembolism has not been identified. Recently, a study from Hong Kong reported the lack of resistance to activated protein C and factor V Leiden among 293 Hong Kong Chinese blood donors(14). Similarly, none of the 114 unrelated, healthy, Thai volunteers revealed factor V Leiden(15). Although we expanded the sample size, a negative result was obtained. In order to obtain an exact figure of the prevalence in the Thai population, a larger sample size should be included.

Recently, two out of 14 Thai adult patients with venous thrombosis were found to have resistance to activated protein C and heterozygotes for factor V Leiden(15). However, 18 children with thromboembolism in this study revealed a negative result. Other new mutations which differ from the western population may be the contributing factors. Additionally, a larger sample size of patients with thromboembolism is required for further study.

Thus, the lack of factor V Leiden is one of the important factors contributing to the low incidence of thromboembolism in the Thai population.

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อุบัติการณ์ของ Factor V Leiden ในผู้บริจาคโลหิตของไทย

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การศึกษาในประเทศทางตะวันตกพบว่าความผิดปกติของยีนที่ควบคุมการสร้าง factor V ที่เรียกว่า "factor V Leiden" เป็นสาเหตุภาวะธร็อมโบสิสทางพันธุกรรมที่พบได้บ่อยที่สุด ผู้วิจัยจึงได้ศึกษาหา prevalence ของ factor V Leiden ในผู้บริจาคเลือดของไทย โดยศึกษาจาก DNA จากเลือดของผู้บริจาคเลือดที่คลังเลือดโรงพยาบาลรามาธิบดีจำนวน 500 คน (ชาย 285 คน, หญิง 215 คน) มีอายุระหว่าง 18 ถึง 60 ปี โดยมีอายุเฉลี่ยเท่ากับ 33 ปี ผู้บริจาคเลือดทั้งหมดเป็นผู้ที่มีสุขภาพแข็งแรงและเข้าเกณฑ์มาตรฐานของ American Association of Blood Banks และไม่เคยมีประวัติธร็อมโบสิสมาก่อน ผลการศึกษาไม่พบ factor V Leiden ใน 1,000 โครโมโซมของผู้บริจาคเลือดทั้ง 500 คน การศึกษานี้แสดงว่าอุบัติการณ์ของ factor V Leiden ในประชากรไทยต่ำมาก และไม่ใช่สาเหตุของภาวะธร็อมโบสิสทางพันธุกรรมในคนไทย

คำสำคัญ : แฟคเตอร์ V Leiden, ผู้บริจาคโลหิตไทย

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