Laboratory Approach in Thai Patients with Venous Thrombosis

Pasra Arnutti MSc*,
Oytip Nathalang PhD*, Wichai Prayoonwiwat MD**,
Rachata Lumkul MD***, Chatuporn Pulket MSc**

** Department of Pathology, Phramongkutklao College of Medicine*
*** Department of Medicine, Phramongkutklao College of Medicine*
**** Department of Pediatrics, Phramongkutklao Hospital Bangkok, Thailand*

This retrospective study aimed to analyze laboratory findings in Thai patients with venous thrombosis in Phramongkutklao Hospital from August 1997 to October 2004. Blood samples obtained from 166 patients with ages ranging from 10 months to 87 years were tested for protein S (PS), protein C (PC), antithrombin (AT), factor V Leiden (FVL) and prothrombin G20210A. It was found that low levels of PS, PC, and AT were observed in 23 patients (13.9%), 21 patients (12.7%) and 11 patients (6.6%), respectively. The incidence of combined low levels of anticoagulant factors occurred in 23 patients (13.9%). Three patients (1.8%) were positive for FVL. All patients were negative for prothrombin G20210A. Additionally, 85 patients (51.2%) were negative for all tests. In conclusion, it is recommended that the screening tests for anticoagulant factors PS, PC and AT be used to investigate the causes of thrombosis in Asian populations due to their cost-effectiveness. However, the detection of gene mutations inducing thrombosis should be considered.

Keywords: *Screening test, Thai, Thrombosis*

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Venous thromboembolism (VTE) is one of the most frequent multicausal diseases; more than one risk factor may combine to effect thrombotic risk, with the pathogenesis involving genetic and environmental risk factors. It manifests clinically by deep vein thrombosis (DVT) and pulmonary embolism (PE) leading to death in about 6% of patients[1]. Laboratory investigations that may help to evaluate the risk for individual patients include the measurements of coagulation inhibitors: protein C (PC), protein S (PS) and antithrombin (AT), in plasma assays[2]. Moreover, the search for the factor V Leiden (FVL) mutation by the plasma activated protein C resistance (APC-R) test and prothrombin G20210A are the most common genetic risk factors known to date in Caucasian populations for VTE[3]. FVL is a single point mutation in the factor V gene (guanine to adenine replacement at nucleotide 1691), causing the substitution of arginine 506 by glutamine at the cleavage site of activated

Correspondence to: Arnutti P, Department of Pathology, Phramongkutklao College of Medicine, 315 Rajavithee Rd, Rajthevi, Bangkok 10400, Thailand. Phone/Fax: 0-2354-7791
E - mail address: parnutti@yahoo.com

protein C[4]. Previous studies in Caucasian populations found that the high frequency of FVL was a risk factor of VTE; heterozygous FVL increased the risk of thrombosis 5 to 10 times and 80 times in homozygous FVL[5-6]. Prothrombin G20210A mutation is the gene replacement of guadinine with adenine at position 20210 in the sequence of the 3'-untranslated region of the prothrombin gene. Carriers of this mutation have 30% higher plasma prothrombin levels than non-carriers, and are associated with an increased potential to form thrombin[7]. Previous studies in Thai populations found that the prevalence of FVL and prothrombin G20210A mutation is lower than Caucasian populations[8-10]. However, the prevalence of VTE is influenced by age and ethnics. Both, hereditary factors (FVL, prothrombin G20210A gene mutation, deficiencies of PC, PS or AT) and acquired risk factors such as estrogen replacement, cancer, cardiovascular disease, surgery, trauma, immobility, use of central venous catheters and autoimmune disease such as antiphospholipid syndrome, contribute to VTE. Recent studies reported that the PC and PS levels in thalassemic Thai patients were significantly lower than those of other anticoagulation factors, which may be responsible for the occurrence of thrombosis[11-12]. The aim of this retrospective study was to analyze the laboratory findings in Thai patients with VTE.

Material and Method

Subjects

One hundred and sixty-six patients with thromboembolic complications, who attended the Division of Hematology, Department of Medicine and Department of Pediatrics, Phramongkutklao Hospital, Bangkok, Thailand, from August 1997 to October 2004, were enrolled in the present study. They comprised 79 females and 87 males with ages ranging from 10 months to 87 years with a mean age of 43.5 years.

Method

Blood samples obtained from each patient were tested for PS, PC, AT, FVL and prothrombin G20210A. PS, PC and AT activity were determined by chromogenic assay, Instrumentation Laboratory Company (MA, USA). The normal ranges of natural anticoagulants were based on manufacturers' instructions. Normal ranges of PS, PC and AT were 60-140%, 70-140% and 75-125%, respectively.

Polymerase chain reaction with restriction fragment length polymorphism (PCR-RFLP) was used to detect FVL and prothrombin G20210A mutation. The analysis of both mutations was performed as previously described[9]. Laboratory findings in 166 Thai patients were analyzed by descriptive frequency number and percent affected.

Results

One hundred and sixty-six patients with venous thrombosis were studied. The percentage of laboratory tests is shown in Table 1. It was found that the low levels of PS observed in 23 patients (13.9%) ranged from 12-58%. Twenty-one out of 166 patients (12.7%) had low levels of PC, ranging from 14-69%. Moreover, 11 out of 166 patients (6.6%) had low levels of AT, ranging from 28-74%. The incidence of combined low levels of anticoagulant factors was 23 patients (13.9%), as shown in Table 2. Five patients (21.8%) had low levels of PS and PC (PS = 17-58%; PC = 32-68%). Four patients (17.4%) had low levels of PC and AT (PC = 40-53%; AT = 33-46%). Seven patients (30.4%) had low levels of PS and AT (PS = 18-54%; AT = 16-70%). Seven patients (30.4%) had low levels of PS, PC and AT (PS = 25-57%; PC = 37-68% and AT = 43-73%). Interestingly, 3 out of 166 patients (1.8%) were positive for heterozygous FVL. All patients were negative for prothrombin G20210A mutation, and eighty-five patients (51.2%) were negative for all tests.

Table 1. The frequencies of positive laboratory findings in 166 Thai patient with venous thrombosis

Test	No. of patients	Percent affected
PS	23	13.9
PC	21	12.7
AT	11	6.6
Combined low level of PC+PS+AT or PS+AT or PC+PS or PC+AT	23	13.9
FVL	3	1.8
Prothrombin G20210A	0	0
Negative for all tests	85	51.2
Total	166	100.0

Table 2. Combined low levels of anticoagulant factors in 23 patients with venous thrombosis

Test	No. of patients	Percent affected
PS + PC	5	21.8
PC + AT	4	17.4
PS + AT	7	30.4
PC+PS+AT	7	30.4

Discussion

Venous thrombosis is a multifactorial disease. Multiple interactions between genetic and environmental factors contribute to the development of the disease. Several genetic risk factors for venous thrombosis have been identified such as PS deficiency, PC deficiency, AT deficiency, FVL and prothrombin G20210A. The deficiency of PS, PC or AT may be congenital or acquired[13-14]. However, the frequency of the deficiencies of naturally occurring anticoagulants in the general population is low, being altogether less than 1%[7]. The low level of PS, PC or AT in patients with alteration of the coagulation factors is a secondary cause to the underlying diseases rather than the hereditary deficiency[15]. Acquired or environmental risk factors include age, immobilization, surgery, trauma, use of oral contraceptives and hormone replacement therapy, pregnancy, puerperium and malignancies[16].

In the present study, it was found that 26.6% of the patients with VTE had low levels of PC and PS, which is similar to previous studies in thalassemic patients[11-12]. In a recent study in β-thalassemia/hemoglobin E patients, the levels of AT were higher in splenectomized patients than non-splenectomized patients and normal control groups[17]. However, in patients with VTE, the prevalence of low levels of AT was only 6.6%. This may be due to the different groups of patients studied. Additionally, the prevalence of combination of low levels of anticoagulation factors was high in the present study. Although the prevalence of FVL and prothrombin G20210A mutations is low in Thai populations compared with Caucasian populations, the prevalence of heterozygous FVL in Thai patients with VTE was 1.8%[3,8-10].

In conclusion, the authors' findings confirm that the alteration of anticoagulation factors is commonly found in Thai patients with VTE. Thus, for patients who present with thrombosis initially, it is recommended that the screening tests for PS, PC and AT be used to investigate the causes of thromboembolism because they are cost effective. However, the detection of gene mutations inducing thrombosis should be considered.

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การตรวจทางห้องปฏิบัติการในผู้ป่วยคนไทยที่มีภาวะหลอดเลือดดำอุดตัน

ภัสรา อาณัติ, อ้อยทิพย์ ณ ถลาง, วิชัย ประยูรวิวัฒน์, รัชฎะ ลำกูล, จตุพร พูลเกษ

การศึกษาครั้งนี้เพื่อต้องการวิเคราะห์ผลการตรวจทางห้องปฏิบัติการที่พบในผู้ป่วยคนไทยที่มีภาวะหลอดเลือดดำอุดตันซึ่งมาเข้ารับการรักษาที่หน่วยโลหิตวิทยา กองอายุรกรรม และกองกุมารเวชกรรม โรงพยาบาลพระมงกุฎเกล้า ตั้งแต่เดือนสิงหาคม พ.ศ. 2543 ถึงเดือนสิงหาคม พ.ศ. 2547 จำนวน 166 ราย มีอายุตั้งแต่ 10 เดือน ถึง 87 ปี จากผลการตรวจทางห้องปฏิบัติการพบว่าผู้ป่วย 23 ราย (13.9%) มีปัจจัยร่วมของสารต้านการแข็งตัวของเลือดต่ำกว่าปกติ (combined low levels of multianticoagulant factors) มีปริมาณ protein S ต่ำ 23 ราย (13.9%), ปริมาณ protein C ต่ำ 21 ราย (12.7%) ปริมาณ antithrombin ต่ำ 11 ราย (6.6%) และพบ heterozygous factor V Leiden 3 ราย (1.8%) นอกจากนี้มีผู้ป่วย 85 ราย (51.2%) ที่ตรวจไม่พบทั้งความผิดปกติของสารต้านการแข็งตัวของเลือด รวมทั้ง factor V Leiden และ prothrombin G20210A

ผลที่ได้จากการศึกษาครั้งนี้คือในผู้ป่วยคนไทยที่มีภาวะหลอดเลือดดำอุดตันนั้น สาเหตุสำคัญส่วนใหญ่มาจากความเปลี่ยนแปลงของปัจจัยของสารต้านการแข็งตัวของเลือดมากกว่าการเปลี่ยนแปลงของกรดอะมิโนในจีน เช่น factor V Leiden หรือ prothrombin G20210A ดังนั้นเพื่อเป็นการลดค่าใช้จ่ายสำหรับผู้ป่วยเหล่านี้ ควรทดสอบเบื้องต้นก่อน เช่น การทดสอบหาปริมาณ protein S, protein C และ antithrombin เพื่อใช้ในการวินิจฉัยหาสาเหตุ แต่อย่างไรก็ตามการตรวจการเปลี่ยนแปลงของกรดอะมิโนในจีนก็ยังจำเป็นที่จะนำมาใช้ประกอบในการพิจารณาร่วมด้วย