Protein S Deficiency is Common in a Healthy Thai Population

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Currently, venous thromboembolism is a growing menace in Asians, approaching to that of Western countries. The most common genetic mutations causing thrombosis in Caucasians are factor V Leiden and prothrombin mutation. However, both are very rare in Asians. On the other hand, natural anticoagulant protein (protein S, protein C and antithrombin) deficiencies are more common in Asian than in Western thrombotic patients. The prevalence of these deficiencies is very low in healthy Caucasians (0.02-0.3%). It is possible that the prevalence is higher in an Asian general population. However, there have been very few prevalence studies to prove this hypothesis.

Protein S deficiency was found in 3.7% (13/352, 95% confident interval 1.72-5.66) healthy Thais. Seven of them were type III deficiency. Similar to previous studies, total and free protein S levels were lower in females, but positively and negatively correlated with age, respectively. In contrast, one protein C deficiency (0.27%, 1/370) and no antithrombin deficiency (0/206) were detectable in our population. Furthermore, the authors found that antithrombin was significantly lower in women and there was a positive correlation between protein C activity and age.

In conclusion, protein S deficiency is more common in Thais than in Caucasians. This result remains to be confirmed by a large population-based study.

Keywords: *Protein S, Protein C, Antithrombin, Venous thrombosis, Asian*

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Venous thrombosis inflicts significant morbidity and mortality on Caucasian population with a published annual incidence over 100 per 100 000 population[1]. However, previous studies in Thai population clearly demonstrated that the incidence of venous thromboembolism is much lower in Asian people[2,3]. The disparity has previously been attributed to the difference in genetic background. Nevertheless, current trends of deep venous thrombosis (DVT) and pulmonary embolism have been increasingly common in Asia. More recent studies on post-operative thrombosis in Malaysians[4], Koreans[5] and Chinese[6] revealed that the incidences of DVT were much higher than previous reports. Therefore, environmental factors probably play an important role. Changing to Western diet and/or lifestyles may predispose them to thrombosis. In addition, these data suggest that the genetic propensity to thrombosis in Asians may be comparable to that of Caucasians. The most common genetic mutations to venous thromboembolism in Western countries are factor V Leiden and prothrombin mutation with the reported rate of 1-2% in general population. In contrast, the prevalence of these polymorphisms is extremely rare in Asians[7]. The most common genetic causes DVT in Asians were protein S (PS), protein C (PC) and antithrombin (AT) deficiency, attributing to over 30% of Chinese thrombophilic patients compared with 7% in Western studies[8-11]. Most of these published studies, as well as unpublished data from Thailand (personal communications), showed that PS deficiency was the most common, followed by PC and AT deficiency, respectively. These natural anticoagulant deficiency may be more prevalent in general Asian population. Alternatively, the high proportion of anticoagulant deficiency may be merely due to the lack of factor V Leiden and prothrombin mutation in Asians.

PS is a vitamin-K dependent cofactor of activated protein C in degradation of factor Va and VIIIa.

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Subjects with familial deficiency of PC or PS have 50% chance to develop thrombosis by the age of 45 years[11]. PS circulates in plasma in either the active free form or the inactive form bound with C4 Binding Protein (C4BP). C4BP is an acute phase reactant. Therefore, changes in total protein S (tPS) may only reflect the alterations in the binding protein. Free PS (fPS) level determination is required to represent plasma PS activity. Three types of deficiency have been described. Type I, which is found in approximately two-thirds of the patients, is characterized by low levels of total and free PS, as well as its function. One-third has type III deficiency with normal levels of tPS, but decreases in fPS. Lastly, type II deficiency, which is rare, is identified by a decrease in PS function, but normal antigen levels[12]. Due to the rarity of type II deficiency and inherent technical problems in measuring PS activity, free PS antigen is a standard method in diagnosis of PS deficiency[12].

Large population-based studies in Western countries included over 10 000 subjects have shown that the prevalences of PC and AT deficiency in general population were very rare. The reported incidences were 0.3% and 0.02%, respectively[13]. Another study in Western Scotland has shown that the prevalence of PS deficiency was between 0.03%-0.13% in 3788 healthy blood donors[14]. A population-based study in Suita, Japan, revealed the PC and AT deficiency prevalence comparable with those of Caucasians[15]. On the other hand, the prevalence of PS deficiency was as high as 1.12% in 2690 Japanese blood donors[16]. This is probably explained by the Lys155Glu substitution in PS molecule that can be found in 1 out of 182 Japanese[17] population and causes lower protein S activity[18]. The prevalence of PS deficiency has never been explored in other Asian countries. The present report is a pilot study on PS, PC and AT levels in Thai population that has a different genetic background from the Japanese. The data will give us deeper insight in the pathogenesis of genetic thrombophilia in Asian countries and knowledge for interpretation of these assays in Thai patients.

Material and Method

Population

Four hundred and thirty six subjects were included in the present study, including 221 healthy blood donors at the National Blood Center, Thai Red Cross Society, and 215 volunteers who were healthy medical students and medical personnel at Chulalongkorn University hospital. All subjects were asked for and all denied any known history of bleeding or thrombotic disorders. Blood was collected and immediately anti-coagulated in 3.2% buffered sodium citrate. Plasma was separated, aliquot within 3 hours after collection, snap frozen and stored at –80 ºC until test.

Method

Total protein S (tPS) and protein C (PC) were measured by sandwich ELISA according to Bertina[19]. Briefly, microtiter plates were coated with 5 µg/ml anti-PS (DAKO A0384, Glostrup, Denmark) or 10 µg/ml anti-PC (DAKO A0370) for 18 hours at 4 ºC, washed and incubated with suitable dilutions of samples, standards or controls overnight. After washing, 0.43 µg/ml HRP-conjugated anti-PS (DAKO P0419) or 3.5 µg/ml anti-PC (DAKO P0374) was incubated for 2 hours. Chromogenic substrate, OPD (Sigma) with H_2O_2, was then added. Finally, the absorbance at 492 nm was read for concentration calculation using standard curves. For the free protein S (fPS) assay, C4BP-bound PS was precipitated with 15% polyethylene glycol 8000 (Sigma) at 4 ºC for 30 minutes and centrifuged. The supernatant was then used for PS ELISA as described above. PC and antithrombin (AT) activity was determined using, respectively, Berichrom AT and Berichrom PC reagents (Dade Behring, Germany), according to the manufacturer's instructions.

Two international Standard Plasmas (Dade Behring, Germany) were used for quality control. An international normal control (lot 502740) and an abnormal control (lot 512676) were performed in every run. Both values needed to be within the company-defined ranges to assure acceptable results. Each sample, and each control was tested in duplicate. Disparate results between the pairs required repeating. The inter-assay coefficients of variation (CV) for fPS, tPS, PC antigen, PC activity and AT activity were 11.36%, 12.20%, 5.63%, 4.96% and 3.49%, respectively.

Data were expressed as means ± standard deviation (SD) for descriptive purposes. The Student's t test was used for comparison of the means. Pearson correlation coefficients were utilized to examine continuous data correlation. All statistical calculations were performed using the SPSS 9.0 for window software. A p-value < 0.05 was considered to be statistical significant.

Results

Three hundred and fifty two healthy subjects had free protein S (fPS) level measurements. Two hundred and twenty one were blood donors and the rest were healthy volunteers. Forty three percent were

male. The mean age was 32.75 ± 10.66 (mean ± SD) years, ranging from 18 to 76 years. The percentage of A, B, O and AB blood group was 17.1%, 32.7%, 46.1% and 8.6%, respectively. The mean fPS level was 84.75 ± 20.68 %, ranging from 32 to 168%. The analysis of the distribution has shown a bell-shape of normal distribution (kurtosis ± 2 SE crosses zero). However, there was skewness to lower values (positive skewness). Three hundred and thirty three individuals had total protein S (tPS) determinations. The average tPS level was 87.48 ± 23.94 %, ranging from 33 to 198%.

Both tPS and fPS levels were significantly lower in females (Table 1). Levels of tPS increased but fPS decreased with advancing age (Table 2). The average fPS of subjects below 45 years of age and 45 years or older were 88.79 ± 24.1% (n=288) and 79.28 ± 21.5%, (n=54) respectively (p=0.007). However, there was no difference in tPS or fPS levels among ABO blood groups. The cut-off point for PS level has been defined variably, depending on laboratories. Different cut-off points have been proposed according to sex and hormonal status. For fPS, they are 65%, 53% and 47% for male, female and females taking oral contraception, respectively.[12] The respective values for tPS are 78%, 66%, 53%. Because the information regarding contraception was not available, the lowest values for female, 47 for fPS and 53 for tPS, were used. Using these cut-off points, Thirteen out of 352 (3.69%, 95% confident interval 1.72-5.66) had low fPS levels, as shown in Table 3. Six also had low tPS (46.2%). Protein C (PC) antigen and activity were measured in 370 and 133 subjects with the mean values of 110.0 ± 25.9 % (52-215%) and 113.8 ± 23.5% (60-185%), respectively. Both levels were significantly correlated. In addition, a positive correlation between PC antigen levels and fPS, as well as tPS, was detectable (Table2). There was no difference in PC between sexes and among blood groups (Table 1). Interestingly, PC activity also increased with age (Table 2). There were 2 subject with low PC antigen (below 60%). One showed PC activity of 60% (low) and the other was 73% (normal), giving the prevalence of PC deficiency of 0.27% (1/370).

Antithrombin (AT) activity was measured in 206 normal subjects. The mean level of the whole group was 102.3 ± 9.42%, ranging from 75-130%. Females showed significantly lower antithrombin activity, compared with male. However, there was no correlation with age. There was no subject with low AT (below 70%) found in the present study. Positive correlations of antithrombin with protein C activity, protein C antigen and free protein S were statistically significant (Table 2).

Table 1. Differences in natural anticoagulant proteins between sexes

		N	Mean ± SD	p values
Protein C function	Male	60	116.4 ± 24.7	NS
	Female	70	111.0 ± 22.4	
Protein C Antigen	Male	162	109.9 ± 27.6	NS
	Female	191	109.3 ± 24.8	
Free protein S	Male	151	95.16 ± 24.5	<0.001
	Female	197	81.62 ± 22.0	
Total protein S	Male	145	110.4 ± 28.5	0.021
	Female	172	103.2 ± 26.5	
Antithrombin function	Male	91	104.0 ± 9.56	0.001
	Female	99	99.56 ± 8.44	

NS = Not significant

Table 2. Correlation coefficient between anticoagulant proteins and age

	Age	PC function	PC Ag	Free PS	Total PS
Age					
PC function	0.299**				
PC Ag	0.100	0.783**			
Free PS	- 0.117*	0.121	0.160**		
Total PS	0.160**	0.366**	0.298**	0.455**	
Antithrombin	- 0.053	0.218*	0.153*	0.153*	0.018

PC = Protein C, PS = Protein S *p<0.05 and ** p<0.01

Table 3. Age, sex, protein C, S and antithrombin levels in 13 cases with low free protein S levels

Case	Free PS	Age	Sex	Protein C Ag	Total PS	Antithrombin
1	32	46	Male	130	58*	103
2	37	21	Female	94	33*	97
3	41	40	Male	105	81	90
4	43	28	Female	91	83	NA
5	43	59	Female	85	114	99
6	45	21	Female	92	60	NA
7	46	33	Female	91	110	107
8	49	20	Male	108	55*	NA
9	51	20	Male	115	58*	NA
10	52	21	Male	63	73*	NA
11	52	32	Male	130	110	NA
12	57	21	Male	71	50*	NA
13	62	26	Male	60	93	NA

NA = not available *Low Total PS values

Discussion

Thrombophilia is a polygenic condition caused by a combination of various genetic susceptibilities.[13] One genetic defect is typically a weak risk factor. However, multiple defects synergistically predispose a patient to venous thrombosis. Different from those of Western countries, deficiencies of natural anticoagulant are the most important causes of genetic thrombophilia in Asia. Therefore, the present study has provided the baseline data in an Asian population for calculation of relative risk of thrombosis. Furthermore, correlations with age and sex of the subjects will be helpful in the interpretations of the laboratory investigations of thrombophilias in Thais.

In the present preliminary study, the prevalence of PS deficiency is 3.7%, which is much higher than that of Western countries. The cut-off levels of anticoagulant protein have been defined variably. In cases with borderline values, the levels may be normalized on repeated assays. Because repeated blood samplings were not available in the present study, the lowest cut-off values were used to assure the deficiency states. None of the PS-deficient subjects had low PC or AT. Therefore, the quality of plasma was likely to be acceptable. Furthermore, only 1 PC deficiency (0.27%) and no AT deficiency was found in the present study, suggesting that these conditions were uncommon in Thais. These data can explain the fact that PS deficiency is the most common hereditary thrombophilia in Thai patients. Interestingly, less than half of subjects with low fPS displayed concomitantly depressed tPS, suggesting that most common deficiencies were type III. Zoller et al. has demonstrated that most of type III PS deficiency were type I deficiency with age-dependent increases in C4BP that normalizes tPS levels.[20] On the other hand, Borgel et al. has found a point mutation, Ser 460 Pro, in the C4BP-binding domain of PS in patients with type III deficiency[21]. The genetic mutations causing this defect in Thais remain to be determined.

Consistent with previous studies in Caucasians, protein S levels both total and free forms were lower in women, suggesting the controlling role of female hormone[12]. The age-related increases in total, but decreases in free PS were probably due to the increases in C4BP with age[14]. Liberti et al has reported that the female hormonal status was responsible for this age-related change in PS[22]. However, a subset analysis of our study showed that men 45 years old or older had significantly lower fPS than those of younger ($81.05\% \pm 19.6$ vs 96.86 ± 24.2, $p = 0.007$). The respective difference in female was not statistical significant ($78.31\% \pm 22.6$ vs 82.25 ± 22.0). Therefore, our data do not support this notion.

In this study, we have found that PC activity was also positively correlated with age. In addition, AT activity was higher in male compared with female subjects. Although this difference was smaller than those of PS levels, it was statistically significant due to the narrow distribution (small SD) of AT activity in both sexes. These findings have never been described in studies in Caucasians. Whether it is unique to Asians remain to be confirmed in larger studies. Furthermore, there were weak, yet significant, positive correlations among these 3 natural anticoagulants. The cause underlying this finding also needs more investigations.

In conclusion, we have found that the prevalence of PS deficiency was more common in Thais

than those of Western countries. However, the PC and AT deficiencies were rare in healthy population. These data need to be confirmed in a larger study. The genetic basis of PS deficiency in Thais is now being investigated.

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ภาวะขาด Protein S พบได้บ่อยในประชากรไทยปกติ

เบญจพร อัคควัฒน์, พลภัทร โรจน์นครินทร์

ภาวะหลอดเลือดดำอุดตันเป็นปัญหาที่พบมากขึ้นเรื่อยๆ ในเอเชียโดยอุบัติการณ์เริ่มใกล้เคียงกับชาวตะวันตก ซึ่งสาเหตุทางพันธุกรรมที่พบบ่อยในคนผิวขาวคือ factor V leiden และ prothrombin mutation แต่ภาวะนี้พบน้อยมากในชาวเอเชีย ในทางกลับกัน ภาวะขาด protein S, protein C และ antithrombin ซึ่งเป็นสาเหตุของภาวะหลอดเลือดดำอุดตันในชาวเอเชียพบได้บ่อยกว่าชาวตะวันตก ภาวะขาดโปรตีนนี้พบได้น้อยมากในประชากรผิวขาว (0.02-0.3%) จึงเป็นไปได้ว่าการขาดโปรตีนเหล่านี้อาจพบในประชากรเอเชียได้บ่อยกว่าชาวตะวันตก

ภาวะขาด Protein S พบ ถึง 3.7% (13 ใน 352, 95% ขอบเขตความเชื่อมั่น 1.72-5.66%) ของอาสาสมัครไทย โดย 7 ใน 13 รายเป็นการขาดชนิดที่ 3 พบว่าค่า Protein S รวม และ Protein S อิสระในเพศหญิงจะต่ำกว่าในเพศชาย และ เมื่ออายุมากขึ้นค่า Protein S รวมสูงขึ้นแต่ Protein S อิสระจะต่ำลง เหมือนกับการศึกษาที่ผ่านมาในชาวตะวันตก นอกจากนี้พบการขาด Protein C เพียง 1 ราย (0.27%, 1 ใน 370 คน) และไม่พบการขาดantithrombin (0 ใน 206 คน) ในคนไทย และพบว่าระดับ antithrombin ในเพศหญิงจะต่ำกว่าเพศชาย ส่วนหน้าที่ของ Protein C พบว่ามีการเพิ่มขึ้นตามอายุด้วย

โดยสรุปภาวะขาด Protein S พบในคนไทยได้บ่อยกว่าชาวตะวันตก ซึ่งต้องรอการศึกษายืนยันในประชากรจำนวนมากต่อไป