ACTIVATED PROTEIN C RESISTANCE INCREASES THE RISK OF VENOUS THROMBOSIS: A PROSPECTIVE STUDY IN 104 PATIENTS WITH UNEXPLAINED THROMBOSIS

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ÖZET

Aktif Protein C Rezistansı Venöz Tromboz Riskini Artırır: Açıklanamayan Trombozlu 104 Hastada Prospektif Bir Çalışma

Aktive olmuş protein C rezistansı (APC), venöz tromboz için oldukça sık görülen kalıtımsal bir risk faktörüdür. Son zamanlarda bu kalıtımsal anormallik, artmış venöz tromboz riski ile ilişkili ana genetik bozukluk olabileceği değerlendirilmektedir. Bu çalışmada, ortalama yaşları 35 olan derin ven trombozlu (DVT) 104 hastada aktive protein C rezistansı fenotipleri belirlendi. Antitrombin III (AT III) gibi Protein C (PC) ve Protein S (PS) aktiviteleride saptandı. Yaş ve cinsiyet bakımından hasta grubu ile uyumlu 110 sağlıklı bireyden, kontrol grubu oluşturuldu. Aktive protein C rezistans pozitifliği, derin ven trombozlu hastalarda ve kontrol grubunda, sırası ile %33.6 ve %4.5 bulundu (p<0.001). Literatür bilgilerine paralel olarak, ailesinde venöz tromboz hikayesi bulunan genç hastalarda aktive protein C rezistansı sıklığının yüksek olduğu belirlendi.

Anahtar Kelimeler: *Aktive Protein C Rezistansı, Faktör V Mutasyonu, Derin Ven trombozu, Pulmoner Embolizm (PE)*

SUMMARY

Resistance to activated protein C (APC) is fairly common inherited risk factor for venous thrombosis. The inherited abnormality is recently being considered to be a major hereditary disorder associated with elevated risk of venous thrombosis. In this study we determined the activated protein C resistance in 104 patients with deep vein thrombosis (DVT) with a mean age of 35 years. Protein C (PC) and Protein S (PS) activities as well as antithrombin III (ATIII) levels were also determined. Control group was comprised of 110 healthy, age and sex matched individuals. Activated protein C resistance positivity was determined as 33.6% and 4.5% (p<0.001) in deep vein thrombosis patients and control group, respectively. In parallel to light of the medical literature information, high prevalence of activated protein C resistance among young persons with history of venous thrombosis was re-emphasized.

Key Words: *Activated Protein C Resistance, Factor V Mutation, Deep Vein Thrombosis, Thrombophilia, Pulmonary Embolism (PE).*

INTRODUCTION

A key component in the anticoagulant pathway is protein C, the zymogen of vitamin K-dependent serine protease. Protein C is activated on endothelial cells by thrombin bound to thrombomodulin. Activated protein C (APC) exerts its inhibitory action by proteolytic cleavage of the precoagulant proteins factor Va and factor VIIIa. Protein S function as a cofactor in this reaction (Fig.1) (1,2).

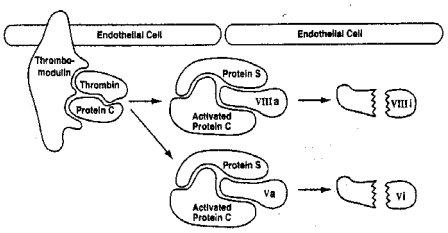

Figure - 1: The protein C pathway. Protein C is activated through interaction with the thrombin-thrombomodulin complex. Activated protein C, in conjunction with cofactor protein S, protoelytically degrades procoagulant factors VIIIa and Va to their inactive counterparts VIIIi and Vi.

Thrombin generated during activation of coagulation functions to convert fibrinogen to fibrin at sites of vessel injury. In surrounding intact vessels, thrombin functions in an entirely different capacity

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by activating the protein C pathway. Thrombin does so by binding to the endothelial cell transmembrane protein, thrombomodulin. Binding of thrombin to thrombomodulin converts thrombin from a procoagulant into an anticoagulant protease that cleaves and activates circulating protein C. APC, in conjunction with protein S as cofactor, inactivates membrane bound factors Va and VIIIa through limited proteolysis. Because of its ability to inactivate two major procoagulants (factors Va and VIIIa), the protein C pathway (with APC as its mediator) is a critical regulatory mechanism that limits clot formation and contributes to the maintenance of intravascular fluidity under normal physiological circumstances.

The genetic defects previously known to be associated with thrombophilia were deficiencies of protein C, protein S, antithrombin III, and dysfibrinogenemia through together. (2,3) Recently, inherited resistance to the anticoagulant action of activated protein C (APC) was found to be a factor involved in thrombophilia, the phenomenon of resistance to APC was first reported by Dahlback et al in 1993. It is defined as a poor anticoagulant response of plasma to APC and is associated with an increase risk of thrombosis.(3) In 1994 it was found that APC resistance is almost always associated with the presence of a mutation in one of the APC cleavage sites (Arg506) of factor V (factor V Leiden). APC resistance caused by the factor V Leiden mutation is a common and strong risk factor for venous thrombosis.(4,5,6,7) Certain acquried conditions, such as pregnancy and oral contraceptive use, may account for a reduced response to APC.(8, 9,10) In family studies and in case-control studies it has been observed that thrombotic individuals without the factor V Leiden mutation have lower APC ratios than nonthrombotic subjects.(10,11,12) These observations suggest that a reduced response to APC may be a risk factor for venous thrombosis and that the APC sensitivity ratio might constitute a useful clinical variable.

We investigated the clinical significance of poor response to APC, and investigated the influence of certain variables (Protein C, Protein S and antithrombin III) on the pathogenesis of thrombosis in otherwise healthy 104 patients with deep vein thrombosis. The result of the patient group is compared to that of age and sex matched healthy controls.

PATIENTS AND METHODS

Selection of patients and control subjects:

This prospective study comprise of 104 patients referred with an unexplained deep vein thrombosis (DVT) and pulmonary embolism (PE) was carried out in King Hussein Medical Center. Patients with known malignant disorders were excluded. Blood samples of all patients were obtained prior to prescribing any medication (including antiaggregants and anticoagulants). The mean age of the cohort was 35 years old (range 20-50). The ratio of male to female subjects was 1:1.5.

Age (±5 years) and sex matched 110 individuals recruited from the volunteer hospital staff, with normal ATIII, PC, and PS levels and without any history of personal and / or familial thrombotic events constituted the control group.

Blood Collection and Laboratory analysis.

Blood samples were withdrawn into evacuated tubes containing 0.106 mmol/L trisodium citrate. Plasma was prepared by centrifugation for 20 minutes at 2,000 g at room temperature to obtain platelet- poor plasma prior to the determinations.

Plasma assays

Total PS levels were determined by Assera-Plate.kit (ELISA method, Stago Diagnostica, Asnıeres-Sur-Seıne, France) utilizing laurel rocket techniques , Functional ATIII was determined by Chromostrate ATIII (Choromogenic assay, Organon Teknika,Boxtel, The Netherlands). Functional PC was determined by Stachrom Protein C (Choromogenic assay, Stago Diagnostica, Asnıeres-Sur-Seıne, France). The manufacturers instructions were strictly followed. The coagulation time was recorded on Sysmex CA-1000 automated Coagulation Analyzer (Sysmex Corporation, Long Grove, IL). The APC resistance test was based on prolong APTT (activated partial prothrombin time) in presence of APC by clotting method, this presence of abnormal of F V leiden is prone to thrombosis.(2,3) The mean Protein C sensitivity ratio (APC-SR) was calculated as through the manufacturers instructions (Stago Diagnostica, Asnıeres-Sur-Seıne, France) . On the other hand normalized APC-SR (n-APC-SR) was calculated as APC-SR value of patient's sample divided by APC-SR value of reference plasma. APC-SR was determined in duplicate for each plasma.

The normal range for n-APC-SR in the leaflet of manufacturer was established to be 0.92 ± 0.14 (mean ± SD). Due to our past experiences we speculated that patients who are heterozygous for the factor V Leiden mutation have an n-APC-SR of 0.64 - 0.78, while those patients who are homozygous for the mutation have an n- APC-SR< 0.64. These results are near to the figures found by Hans et al. in Netherlands.(13)

Statistics were performed by using SPSS and EXCEL for Windows. Statistical data were analyzed with the independent sample t test, and the descriptive analysis and C2 tests. Data are expressed as means ± SD and %. A p-value smaller than 0.05 was considered statistically significant.

RESULTS

Occurrence of thrombosis was seen at markedly younger age in the homozygous individuals in comparison with the other patients; the median age at onset of thrombosis was 28 years old versus 35 years old in the heterozygous and 40 years old in the patients without the mutation (Table I).

Seven (70%) out of 10 homozygous patients were female, compared with 15 (60%) heterozygous and 42 (61%) individuals without the mutation (Table I).

TABLE - I
General Characteristics and Factor V Genotype Estimation of 104 Thrombosis Patients

	Normal n=69	Heterozygous n=25	Homozygous n=10
Age (years)			
Median (Range)	40 (30-50)	35 (25-50)	28 (20-42)
Sex			
male n(%)	27 (39)	10 (40)	3 (30)
female n(%)	42 (61)	15 (60)	7 (70)

n-APC-SR, protein C functional (PCF), PS and ATIII values of patients and control subjects are shown in Table II and Table III. In accordance with these results, n-APC-SR, PCF, PS and ATIII results were statistically significantly lower ($p<0.001$) in thrombotic patients than healthy controls (Table II, Table III, Fig. 2).

TABLE - II
All Results in Patients and Controls

	Normal Ranges	Patients n=104	Control n=110	p
n-APC- SR	<0.78	0.82 ± 0.13	0.92 ± 0.08	<0.001
PCF %	60-140	54.46 ± 20.77	98.30 ± 9.29	<0.001
PS %	60-120	62.04 ± 22.76	100.8 ± 8.59	<0.001
ATIII %	80-120	80.78± 23.78	107.7 ± 9.10	<0.001

Results were expressed as mean±SD

TABLE - III
Number (%) of Patients and Controls With (n-APC-SR, PCF,PS,ATIII) Deficiency

	Normal Ranges	Patients n=104 n(%)	Control n=110 n(%)	p
n-APC- SR	<0.78	35 (33.6)	5 (4.5)	<0.001
PCF %	60-140	75 (72.1)	0 (0)	<0.001
PS %	60-120	69 (66.3)	0 (0)	<0.001
ATIII F %	80-120	36 (34.6)	0 (0)	<0.001

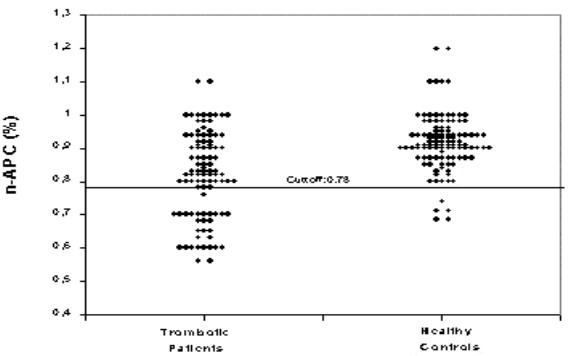

Figure - 2: n-APC-SR values of thrombotic patients and healthy controls.

60% of the patients was found to have recurrent DVT as confirmed by natural history questioner, 10 patients of the DVT group developed PE, 6 females had the first objectively confirmed episode of thrombosis during delivery or post delivery period.15 females were on oral contraceptives.

Number of patients with n-APC-SR value abnormality alone was 7/35 (%20) and number of thrombotic patients with abnormality of all parameters was 12/35 (%34) (Table IV).

TABLE - IV
Percent of Abnormality of the Parameters in Thrombotic Patients and Healthy Controls

	Total number of patients with APC (+) n(%)	Patients with APC (+) and 3 tests* n(%)	2 tests n(%)	1 test n(%)	Only positive APC n(%)
Patients (n=104)	35 (33.6)	12 (11.5)	11 (10.6)	5 (4.8)	7 (6.7)
Control (n=110)	5 (4.5)	-	-	-	5 (4.5)

*the three tests: PC, PS, ATIII.

The correlation between n-APC-SR and PCF, PS, ATIII levels are shown in table V. There were positive significant correlation between n-APC-SR and the other parameters in controls. But no correla-

tion between n-APC-SR and PCF, ATIII except PS levels in patients was found.

TABLE - V
Correlations Result Between n-APC-SR and the Other Parameters

		PCF	PS	ATIII
Patients and control	r	0.416	0.464	0.410
	p	<0.001	<0.001	<0.001
	n	214	214	214
Controls	r	0.363	0.373	0.537
	p	<0.001	<0.001	<0.001
	n	110	110	110
Patients	r	0.088	0.23	0.144
	p	0.377	0.019	0.145
	n	104	104	104

DISCUSSION

APC resistance caused by factor V leiden mutation is a strong risk for venous thrombosis.(1) The prevalence of heterozygocity for factor V leiden in general population is established to be 2-7% (3,7,14).

The phenotypic expression of resistance to APC is characterized by a poor response to the anticoagulant activity of APC, a key enzyme in the down-regulation of blood coagulation, which causes a disposition for hypercoagulable state. The cases with resistance to activated protein C are explained by a point mutation in the gene for coagulation factor V, resulting in replacment of an Arg to Gln at positon 506 (factor V:Q506, often denoted as factor V leiden), one of the three activated protein C cleavage sites in activated factor V. The mutation is inherited as autosomally dominant trait in the general Caucasian population.(3,13,15) A number of clinical studies, using different inclusion criteria, show a prevalence of activated protein C resistance of 20-60% among patients with venous thromboembolism.(16,17) For this reason, laboratories are faced with an increasing number of samples referred for APC resistance diagnosis.

In this study it is confirmed that a reduced n-APC-SR is associated with an increased risk of venous thrombosis; that is resistance to APC was found in 33.6% of 104 patients with DVT and PE. Due to the data shown on Table II and Table III, poor response to APC is a common high risk factor for deep vein thrombosis. The prevalence of resistance to APC in the control group was found to be 4.5%, which is consistent with the figures concerning the general population. Ratio of prevalance of resistance to APC between male/female was found as 1:1.5. This finding may be due to some acquired conditions of high risk of thrombosis concerning the female sex like pregnancy, oral contraceptive use, etc. (10,11).

It was previously demonstrated that homozygous individuals with the factor V leiden mutation have a n-APC-SR value of < 0.64 (n=10), whilst for that parameter heterozygous individuals expressing n-APC-SR 0.64-0.78 (n=25), and non carriers expressing a n-APC-SR of >0.78.

The actual expression of the anticoagulant activity of a fixed amount of APC (and n-APC-SR) might be dependent on the plasma concentration of other coagulation proteins: protein S (as a cofactor of APC), factor V and VIII (as substrates of APC), and other vitamin K dependent proteins. As shown in table IV that the number of patients with only n-APC-SR abnormality alone was 7 (20%) and the number of thrombotic patients with abnormality of all parameters was 12 (34%).

We conclude that APC resistance increase the risk of venous thrombosis, despite the exact diagnosis can be made by the polymerize chain reaction (PCR) technique where the factor V:Q506 mutation site can be identified.(1) n-APC-SR test is conceptually simple and easy to perform in any coagulation laboratory; assuring to be carefully standardized.

Finally, one has to keep in mind that APC-SR should not be reckon upon evaluating the patients with a base line prolongation of the APTT, as the determination of APC-resistance of a plasma sample is based on the prolongation of its APTT in the presence of APC, its APTT without APC being normal(18).

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