
The Prevalence of Activated Protein C Resistance and F V Leiden in Healthy Population of Edirne, Turkey

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ABSTRACT

Activated protein C (APC) resistance has been found to be an important cause of venous thrombosis. The prevalence of F V Leiden (FVL) in general population is variable according to the region and the ethnic group. The aim of this study was to determine the prevalence of APC resistance and FVL in healthy population in Edirne as a representative sample of province of Edirne. Total 467 healthy subjects were studied. There were 238 males (50.96%) and 229 females (49.04%). APC resistance was studied by functional and DNA methods. There were a total 22/476 subjects (4.7%) were APC resistance. There were 20/476 subjects (4.28%) who had FVL by DNA test. Of these, there were 18 heterozygous and 2 homozygous FVL and other two subjects have no FVL mutation but have the high levels of FVIII to explain acquired APC resistance. The coexistence of FVL and the deficiencies of protein C (1/22), protein S (2/22) and antithrombin (1/22) were also studied. No one of subjects had prothrombin gene mutation. The data showed that the prevalence of APC resistance and FVL in healthy Turkish population were similar to the previously reported publications in Turkey and Europe. One thing is a keeping in mind to be the coexistence of FVL and the other known thrombophilic risk factors.

Key Words: Activated protein C resistance, FV Leiden, Prevalence in healthy population, Turkey.

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INTRODUCTION

Thrombosis is an important complication of several inherited and acquired conditions referred to generically as the thrombophilias. The term thrombophilia was coined by Egeberg in 1965 to describe a tendency to thrombosis in a Norwegian family that was shown to have antithrombin III (AT III) deficiency^[1]. Since then, the definition of thrombophilia has been broadened to include any patient with an increased tendency to

thrombosis which may be either inherited or acquired. Patients are considered to be potentially thrombophilic if they have not had an episode of thrombosis but have laboratory abnormalities or clinical disorders that are known to be associated with an increased risk of thrombosis^[2,3].

Antithrombin (AT) deficiency, alterations of the protein C (PC) system, activated protein C (APC) resistance, prothrombin gene mutation, and hyperho-

mocysteinemia are the most frequently recognized causes of hereditary thrombophilia^[4]. Several studies focusing on the laboratory evaluation of patients with thromboembolic disease have been published^[5-7]. However, the reported prevalence of different causes of thrombophilia is highly variable, probably due to the patient selection criteria or to the region and the ethnic group.

APC resistance has been found to be an important cause of venous thrombosis and familial thrombophilia, recently^[2]. Most of the cases of APC resistance described are due to factor V Leiden (FVL). This results from a point mutation in F V gene at position 1691 GÆA converting the arginine (R) 506 in F V cleavage site by APC to glutamine (Q). As a result, the proteolytic inactivation of F V is greatly impaired leading to APC resistance. This APC resistance is associated with high risk of venous thrombosis. The risk is 5-10 fold for heterozygotes and 80 fold for homozygotes^[8,9]. The prevalence of FVL in general population is variable according to the reasons mentioned above. The high prevalence of FVL was reported in Southeast of Europe (9-15%) and the middle east (13%), while it is low in black and Asian population^[9-13].

The population of the Trakya region of Turkey is composed of residents and the immigrants from Western Trakya and the Balkans due to its geographic location as a bridge between Europe and Asia. Because of this reason, the aim of this study was to determine the prevalence of APC resistance and FVL and the combined deficiencies with other known natural inhibitors in healthy population in Edirne, which is located in Trakya region of Turkey, as a representative sample of province of Edirne.

MATERIALS and METHODS

Sampling size of the study was calculated 467 (467/118.575) subjects in accordance with 95% reliability \pm 2 SD and 5% prevalence in the population of Edirne. All healthy persons who were volunteers to get involved into the study did so after filling in forms which interrogated them about their personal health status. After recording the clinical history, blood was obtained from subjects who had no history of thromboembolism, taking medications such as warfarin and transdermal oestrogen. Blood samples were collected

into vacuum tubes containing 0.129 M trisodium citrate. In all subjects prothrombin time (PT), activated partial thromboplastin time (aPTT), F V, F VIII, AT, PC, protein S (PS) and PC sensitivity ratio (APC-SR) were determined by coagulometric methods using commercial kits and ST4 semi-automatic instrument (Diagnostica Stago, France).

APC resistance test: The test was performed according to the instructions of the manufacturer using purified APC from Diagnostica Stago, France. The cut-off value for the APC sensitivity ratio was set at 1.80. The subjects which gave reading between 1.8-2.0 were retested. The subjects whose have less than 1.8 APC-SR were labeled for APC resistance. Among 467 subjects, 22 subjects have APC resistance by functional analysis. Blood samples were collected from these subjects and transferred under cold chain condition to Hacettepe University Division of Pediatric Hematology to make further investigation for FVL and prothrombin gene mutations analysis.

The coexistence of FVL and the deficiencies of other known natural inhibitors such as AT, PC and PS also was searched.

For statistical analysis we used the descriptive, one way Anova, correlation and regression analysis with SPSS software package.

RESULTS

A total of 467 healthy subjected were studied. The mean age of the 467 subjects was 35 ± 17 years, 238 (50.96%) of them were males and 229 of them (49.04%) were females. In the study group, APC-SR was 2.34 ± 0.37 and this was within the normal range. The subjects whose APC-SR was 1.8 and lower were accepted to be functional APC resistant. The frequency of APC resistance was 4.71% (22-476), and that of FVL was 4.28% (20-476). FVL was present in 90.9% of the subjects who had APC resistance by functional assay. Two were homozygous and 18 were heterozygous. Two subjects with APC resistance had no FVL mutation. The high levels of F VIII in those two subjects were found to explain the acquired APC resistance. The combine deficiencies of PC, PS and AT with FVL also were carried out. One of PC deficiency (1/22), two protein S deficiencies (2/22) and one antithrombin deficiency (1/22) were determined. No one of subjects

had prothrombin gene mutation. All of those results were summarized in Table 1. There were a negative correlation among APC-SR and body mass index ($r = -0.98$, $t = -2.121$, $p = 0.034$) and age ($r = -0.92$, $p = 0.04$) but not relationship sex, F VIII and F V levels.

DISCUSSION

Venous thrombosis is a common multifactorial disease, known to be associated with a number of acquired and inherited factors. Dahlback et al described an inherited form of APC resistance, subsequently shown to occur in 20-60% of patients with venous thrombosis and 2-5 % of the general population^[2]. Most of surveys of FVL have been carried out on population of European origin, and Asian population^[9,13]. Based on the published surveys, the prevalence of FVL in general population varies widely from country to country (0.98-15.0%). In Southeast of Europe, the prevalence is high according to other countries^[9-11]. The prevalence of FVL in Turkey and in Greece was reported to be 9.16% and 15.0%, respectively. Until recently, the published surveys from Turkey have small sample size and not an epidemiological study^[14,15]. The aim of this work was determined the prevalence of APC resistance

and FVL in Edirne. The screened population was sufficient enough to represent the target population living in provincial Edirne in terms of size and composition. Most of the APC resistance seen in this survey is due to FVL (90.9%). There were two subjects who showed APC resistance by functional assay but not confirmed by DNA analysis. F VIII levels of two subjects were so high. It may be to explain the acquired APC resistance of two subjects. We could not determine any subject who had negative functional assay-positive genetic analysis.

The results of this study was similar to previously reported studies from Turkey, however lower than the study of Ozbek and Tangun^[10,14,15]. This difference may be explain by sample size and the criteria used to select individuals. The sample size of our study was calculated by systematic random sampling.

It is known that the combination of FVL with the other prothrombotic inherited risk factors in etiopathogenesis of venous thrombosis^[16-19]. To address this issue in our study group, we searched the coexistence of FVL and prothrombin gene mutation, the deficiencies of PC, PS and AT. We could not find any subject who had prothrombin gene mutation. However, one PC, two

Table 1. All results of the studied group

Age (years)	n	X ± SD	Range
0-14	96	10.41 ± 3.07	0.5-14
15-45	245	31.81 ± 6.34	17-45
> 45	126	53.66 ± 7.92	46-78
APC-SR (total)	467	2.54 ± 0.37	1.10-3.63
APC resistant group (functional assay)	22	1.57 ± 0.18	1.10-1.80
FV Leiden	20	1.57 ± 0.18	1.1-1.77
Heterozygous	18 (12 M, 6 FM)	1.61 ± 0.16	1.2-1.77
Homozygous	2 (2 M)	1.3 ± 0.14	1.1-1.4
Acquired APC resistance without FVL	2 (M, 32 and 40 y)		
FV III levels		183.1%	
FVL + PC deficiency % activity	1 (32 y, M)	200%	
FVL + PS deficiency % activity	2 (2 M, 3 and 32 y)	31.3%	
FVL + AT deficiency % activity	1 (M, 24 y)	37.1	
		34.8%	
		56%	

M: Male, FM: Female, APC-SR: Activated protein C sensitivity ratio, PC: Protein C, PS: Protein S, AT: Antithrombin, FVL: Factor V Leiden, F VIII: Factor VIII.

PS and one AT deficiencies were determined. It is interesting that the combined different inherited risk factors were found in healthy population.

In conclusion, the study showed that the prevalence of APC resistance and FVL in healthy subjects in Trakya region of Turkey is similar to European population. There is a good correlation between the functional assay and genetic analysis of APC resistance. The surprising high prevalence of the other hereditary risk factors were determined the combined with APC resistance.

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