PREVALENCE OF RESISTENCE TO ACTIVATED PROTEIN C (APC-resistance) IN BLOOD DONORS IN KOSOVO

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Abstract

One of the most frequent hereditary causes of thrombophilia is, without a doubt, resistance to Activated Protein C (APC-resistance), which is a consequence of point mutation in gene coding for coagulation Factor V (Factor V Leiden) in 90-95% of cases.

The aim of this paper was to determine prevalence of APC-resistance in a group of healthy blood donors. The size of the group is quite representative of Kosovo Albanians.

A total of 944 blood donors were examined (537 males and 407 females), for whom APC-resistance was determined by functional methods of coagulation using the kit ACTICLOT® Protein C Resistance. Method is based on the test of APTT determined twice: first in the presence and second in the absence of activated Protein C (APC). The ratio of these two values constitutes is called Activated Protein C - Sensitivity Ratio (APC-SR).

From 944 examined donors, pathologic values of APC-SR (1,3-1,9) were found in 32 persons (3,4% of the total number). The distribution among sexes was 3,35% (18/537) in male and 3,43% (14/407) in female subjects. The mean values of APC-SR (1,64 in male and 1,71 in female subjects) were not significantly different (P = 0,22).

Based on these results, we conclude that the prevalence of APC resistance in Albanian population of Kosovo is within the lower limit of prevalence in general population in different countries of European countries, which, according to some authors ranges is from 3 to 7%.

KEY WORDS: resistance of APC, FV Leiden, protein C pathway, thrombophilia

Introduction

Venous thromboembolism is considered one of the most frequent causes of morbidity and mortality in western countries. It affects approximately 1 person per 1000 inhabitants (1). Resistant protein C plays an important role in ethiopathogenesis of thromboembolism. Protein C is a vitamin K dependent protein (2). In the presence of cofactor thrombomoduline, thrombin transforms protein C into activated form of protein C (APC) which is a key anticoagulant enzyme, necessary for regulation of coagulation process through inactivation of factors Va and VIIIa (3). Weak anticoagulant response to activated protein C is called resistance to activated protein C (APC- resistance) (4). Phenomenon of APC resistance was described by Dahlbäck et al. for the first time, who have identified a patient with thrombosis (with family history positive for thromboembolism), in whom APTT did not prolong as expected after his plasma was supplemented with exogenous APC (5). Molecular mechanism of APC resistance is well known and at least 90 – 95% cases with APC-resistance phenotype can be explained by point mutation in the gene of factor V. Mutation occurs as a consequence of replacement of guanine with adenine base in position 1691 of nucleotide chain resulting in the replacement of arginine with glutamine in position 506 of factor V molecule (4, 5, 6). Factor V with this mutation is known as FV Leiden or more precisely factor V G1691A (factor V Arg506Gln) (7). Presence of APC resistance increases the risk of recurrent venous thromboembolism (8, 9, 10, 11, 12, 13). The data exist which indicate the possibility of combined presence of factor V Leiden and hereditary deficit of one of physiological inhibitors of coagulation such as antithrombine III, protein C or protein S. This condition is manifested as increased prevalence of venous thrombosis in people with this abnormality (14). Besides mentioned disorders, factor V Leiden can also be accompanied by hyperhomocysteinemia as well as with mutation in gene for prothrombine (G20210A) (15, 16). Even though hypercoagulabile conditions that are a consequence of mentioned genetic disorders occur more frequently as venous thromboembolism there is a plenty of data which indicate thrombosis in the arterial vessels as well (brain infarct, coronary artery disease) in the presence of either isolated, APC-resistance or combined with deficit of native inhibitors of coagulation (antithrombine III, protein C, protein S) or combined with mutation in gene for prothrombine (G20210A) and with hyperhomocisteinemia (17, 18, 19). Comparing with the deficit of physiological inhibitors of the coagulation, which are responsible for 5 – 10% of inherited thrombophilias, resistance to APC can be found in 15 – 60% of patients with different thromboembolic phenomena, depending on the ethnic group and geographic region (4, 11, 20, 21, 22). Activated protein C resistance is also found in healthy individuals with no previous thromboembolic events. Prevalence of APC- resistance in healthy persons differs depending on geographic region, ethnic group or race and cases are usually heterozygous for FV Leiden (23, 24, 25, 26). Numerous factors can provoke thrombosis in people in good health including the defect in factor V. Triggering factors may include different injuries, surgical interventions, obstetrical and pregnancy disorders (27, 28, 29). There are many publications about the prevalence of protein C resistance in healthy persons. Variations of its prevalence in different countries in the world were found to depend on ethnic, racial and geographic background. The aim of this paper was to determine the prevalence of this phenomenon in the Albanian population of Kosovo, as this nation constitutes more than 90% of the total population in this country.

Materials and Methods

APC-resistance was determined in 944 individuals, 537 males and 407 females who donated blood during the year 2007 in The National Blood Transfusion Centre of Kosovo, in Prishtina. Detailed history, physical exam and CBC were performed in each volunteer before blood drawing. The blood taken with sodium citrate 3,2% in 9:1 blood to citrate ratio was centrifuged at 2000 rpm for 15 min to separate plasma which was quick-frozen and maintained at -35 °C till the moment of testing. Resistance to APC was determined by functional test for APC-resistance (ACTICLOT® Protein C Resistance kit, American diagnostics), which is a screening test for APC-resistance. Kit consists of four reagents: R1 (RW-V + APC reagent, Polybrene, Hepes, BSA), R2 (RW-V reagent, Polybrene, Hepes, BSA), R3 (PTA reagent - activator of prothrombine, EDTA, Hepes, BSA) and R4 (plasma diluent). All reagents were in lyophilized state and kept refrigerated between 2 to 8 °C until use. Testing was performed in semi-automatic coagulometer Amelung KC4/10A for APTT test before and after addition of activated protein C. The test results were expressed as sensitive ratio of APC (APC-SR), which represents APTT (+APC)/ APTT (-APC) (12). Quality control of the test was done by positive and negative control, performed during determination of APC-SR

for every series of examined donors. According to this method normal values of APC-SR are 4,2-6,9, while pathologic values (if the cause is the presence of factor V Leiden) differ for heterozygote and homozygote people ranging from 1,3 – 1,9, and 1,0 – 1,1 respectively.

Results

Basic functional test for the detection of resistance to activated protein C was performed in 944 blood donors between 18 and 65 years of age. Of those, 537 (56,9%) were males, and 407 (43,1%) were females. Decreased values of APC-SR (between 1,3 and 1,9) were found in 32 or 3,4% of examinees. Among those 18 (1,9% of total) were male subjects and 14 (1,5%) females (Table 1). Prevalence of APC-resistance for the whole group agrees with the prevalence within groups according to sex, which was 3,4% (rounded to the first decimal for both male and female examinees).

	Examined individuals		**APCr individuals**	
Gender	N	%	N	% of total
M	537	56,9	18	**1,9**
F	407	43,1	14	**1,5**
Total	**944**	**100**	**32**	**3,4**

TABLE 1. Prevalence of APC-resistance and its distribution across sexes

	Males	Females
X	1,64	1,71
N	18	14
SD	0,178	0,124
SEM	0,042	0,033

TABLE 2. Statistic parameters of APC-SR of cases with APC-resistance

Table 2 presents statistic parameters of APC-resistance according to sex. Average values of APC-SR in males with APC-resistance were 1,64, and in females 1,71. The difference between means was not statistically different (t test: p = 0,22, with CI 95 between 0,044 – 0,184).

In Figure 1 presents average APTT values (in seconds) with and without presence of APC and APC-SR of both healthy persons (with normal APC-SR) and those with APC-resistance (with abnormal APC-SR). From the figure it can be clearly seen that in persons with APC resistance, APTT is shortened because of deficient inhibitory effect of APC on factor V as a consequence of genetic mutation.

Distribution of normal and pathological values of APC-SR in female and male examinees is given in Figure 2. It is evident that the decreased (pathologic) values of APC-SR in both male and female groups are lower than 2.

Discussion

The phenomenon when activated protein C (APC) is not successful in inhibition of activated factor V is called resistance to activated protein C (APC-resistance), which was described for the first time by Dahlbäck in 1993 (5). Even though APC-resistance is the most frequent factor implicated in the venous thromboembolism of different localization (5,6,8,9,11,12), today there is ample of data which shows the role of APC-resistance in development of thrombosis in arterial vessels (brain stroke and coronary artery disease) either as

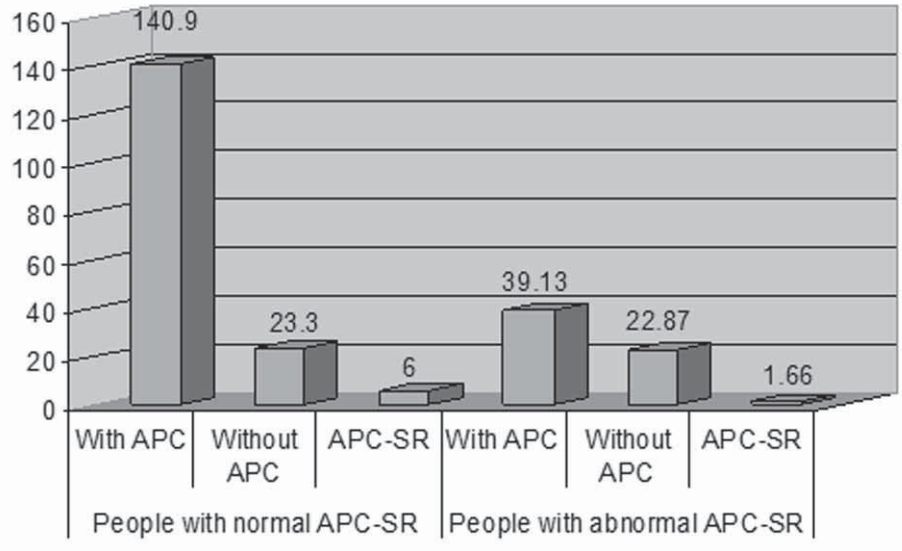

FIGURE 1. Average APTT measured in the presence of APC and APTT without APC and APC-SR in examines with normal and abnormal APC-SR

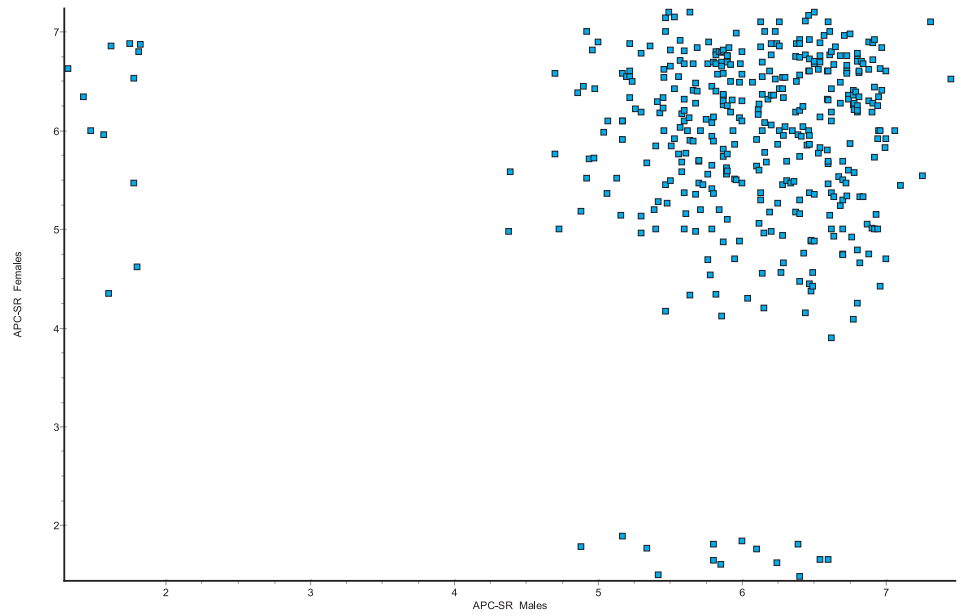

FIGURE 2. Distribution of normal and pathologic values of APC-SR in female and male examinees.

a consequence of its isolated defect (APC-resistance), or as a combined disorder, when APC is associated with deficit of antithrombin III, protein C, protein S or with mutation of gene for prothrombin (G20210) and with hiperhomocisteinemia (15,16,17,18,19,26).

Molecular foundation of APC-resistance lies in amino acid substitution in factor V molecule, where arginine is replaced by glutamine in position 506. Substitution is mediated by point mutation (G to A) in the position 1691 of gene for factor V (4, 5). APC-resistance is also called factor V Leiden or in concise form: factor V G1691A (factor V Arg 506 Gln, factor V Leiden) (7). There is another inherited form of APC-resistance, called F V Cambridge (Arg306-Th) (30). APC-resistance may also appear as an acquired form as a consequence of increased concentration of F VIII in lupus anticoagulant syndrome and pregnancy (31).

This study was motivated by clinical importance of this pathology as well as the fact that this defect can be found in healthy people. The study includes the total of 944 healthy individuals (537 males and 407 females), who voluntarily donated blood in 2007. The number of the examined individuals was sufficiently representative to judge fairly accurately about the prevalence of APC-resistance in the population of Kosovo. All examinees had routine physical and laboratory workup before donating the blood. According to the medical history, no previous thromboembolic events were recorded in any of the individuals. Laboratory workup of examined persons included functional test for APC- resistance done with ACTICLOT® Protein C Resistance kit, American diagnostics (see Material and methods). The evaluation was done by measuring APTT. The tested plasma was diluted with normal diluent plasma in ratio 1,5:1 (30 µL examined plasma: 20 µL diluent plasma) in order to ensure the correction for possible deficit of any other coagulation factor, particularly if the examined persons were on oral anticoagulant therapy (32). Our results show decreased values of APC-SR in 32 persons or 3,4% of all examined persons (32/944). Decreased values of APC-SR were found in 14 females or in 1,5% (14/944) and 18 males or 1,9% (18/944) of total number. We found similar prevalence of decreased values of APC-SR in both sexes: 3,35% in female and 3,44% in male examinees.

Because of lack of equipment, we were not able to specifically determine prevalence of F V Leiden by DNA typing in our cases with APC-resistance. Thus, we cannot be certain of exact prevalence of this genetic disorder. However, indirectly we can speculate on the approximate prevalence based on use of modified test of APTT, because the use of diluent plasma increases the specificity of this test. We believe that there is high credibility that Factor V Leiden is present in over 95% of our cases with APC-resistance or in about 3% of general population. Prevalence of APC-resistance varies in different regions of the World, depending on ethnic, racial and geographic factors. Based on this, many epidemiologic studies were done in different countries and continents. Regarding prevalence of APC – resistance in

healthy persons, our data are much closer to the results of some other authors, where prevalence is from 2 to 5% (11, 33). Factor V Leiden is more frequent among white population. In general population of Europe, point mutation in factor V (FV Leiden) is 3 – 7% (34). Hermann et al (1998), found different percentage of factor V Leiden in several countries of different continents as follows: in Germany 2,9%, Poland 5,0%, Argentina 5,1%, Venezuela 1,6%, Costa Rica 2,0% and India 1,3% (35). In some Asian countries prevalence of Factor V Leiden is similar to those in Europe, as in Turkey 5% (23), Iraq 3% (24), Saudi Arabia 2,5% (36), however in some other Asian countries the prevalence is much higher: for example in Lebanon 14,2%, Syria 13,6% and Jordan 12,25% (37) or much lower as in China, Korea, Taiwan and Japan (35, 38). In healthy population of Canada (blood donors) prevalence of Factor V Leiden is 5,3% (25) and in the USA 5%. Other authors have reported different prevalence of APC-resistance in healthy population of different countries, ranging from 2 to 13% (4).

Conclusion

The prevalence of APC-resistance in general healthy population of Albanian ethnic group in Kosovo is 3,4%. We cannot know the exact percentage of F V Leiden in our cases with APC-resistance, however based on the data from literature we believe that it should be about 3%. This percentage is in the lower limit of prevalence of F V Leiden in Europe. Our results agree with those of other authors for prevalence of APC-resistance outside European countries. Since this disorder can be associated with inherited deficit of inhibitors of coagulation we hope that, in the near future, we would conduct parallel examination of APC-resistance (with PCR) and other inhibitors of coagulation in patients with thromboembolic disorders and healthy persons to determine prevalence of combined defects that are responsible for thrombophilias in the population of Kosovo.

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List of Abbreviations

APC	-	Activated Protein C
APCr	-	Resistance to Activated Protein C
APC-SR	-	Activated Protein C- Sensitivity Ratio
APPT	-	Activated Partial Thromboplastin Time
CBC	-	Complete Blood Count
RPM	-	Revolutions per minute (the number of full rotations completed in one minute)
RVV-V	-	Russell's viper venom- Factor V
BSA	-	Bovine Serum Albumin
PTA	-	Prothrombin Activator
EDTA	-	Ethylene Diamine Tetra Acetate
DNA	-	Deoxyribonucleic Acid
PCR	-	Polymerase Chain Reaction

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