Activated Protein C Resistance in Behçet's Disease

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ABSTRACT

We investigated activated protein C resistance (aPCR) using modified activated partial thromboplastin time (aPTT) in 32 patients with Behçet's disease (BD) and 9 healthy controls. None of the healthy controls were found to have aPCR. However, 11 out of 32 Behçet's patients (34.3%) were found to have aPCR. The frequency of aPCR was increased to 44.4% among 18 Behçet's patients having a history of venous thrombosis. In the subgroup of 14 patients without venous thrombosis, aPCR frequency was %22.2. Our findings show that, besides other factors, aPCR may also predispose patients to venous thrombosis in BD. The detection of aPCR, using modified aPTT may serve as a routine screening test to determine the necessity of prophylactic anticoagulation treatment in patients with BD.

Key Words: Behçet's disease, Activated protein C resistance, Thrombosis.

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INTRODUCTION

Morbidity and mortality in patients with BD are largely affected by arterial and venous thrombosis[1]. Although vasculitis, resulting in endothelial damage, is the main pathogenetic factor, we know that thrombotic development in BD is multifactorial[2]. aPCR is found to be present in 20-60% of patients with idiopathic venous thrombosis[3]. A single point mutation in factor V is the well known cause of aPCR in more than 90% of cases. This mutated factor V is known as factor V; Q506 or factor V Leiden[4]. Due to the structural change in factor V, caused by a point mutation, the inactivation of factor V by activated protein C (aPC) is decreased nearly 10 times. Consequently, the propensity for thrombosis is increased 10 times and 80 times, in those with heterozygote and homozygote mutation, respectively[4,5]. Furthermore, the coincidence of the occurence of aPCR with other inherited and enviromental risk factors is well known for increasing thrombosis formation furthermore. The prevalence of Factor V Leiden mutation shows ethnic differences in various populations. The lowest prevalences have been found in Eskimos, Japanese, Chinese and Africans[4].

aPCR may be investigated by showing Factor V Leiden mutation, using molecular biological methods, as well as coagulometric tests which are widely used. The principle of the coagulometric tests is based on the prolongation of the coagulation time by activated pro-

tein C. Since aPCR may be found even in the absence of a mutation, it has been recommended that molecular biological methods and coagulometric tests should be performed at the same time. In the literature, studies, show the presence of Factor V Leiden mutation in BD. In this study, we investigated aPCR, by using modified aPTT measurements in Behçet's patients with and without venous/arterial thrombosis.

MATERIAL and METHODS

32 patients were followed by the Ege University Rheumatology Department, all fulfilling the criteria of International Study group for Behçet's Disease. Nine healthy controls were included in this study. The demographic features of both groups are given in Table 1.

The exclusion criteria for Behçet's patients were given as follows:

1. The presence of active thrombosis,

2. Pregnancy or puerperium,

3. Oral contraceptive use,

4. Antiphospholipid antibody positivity Investigation of antiphospholipid antibodies was performed by testing IgG and IgM anticardiolipin antibodies (ACA), screening for the presence of lupus anticogulant (LAC) and ordering VLDL. ACA were tested by using commercial ELISA kits (ORGenTec, Diagnostika Gmbh). Values less than 10 GPL and 7 MPL were accepted as negative for IgG and IgM ACA, respectively. Finding a normal basal aPTT, which is a phospholipid dependent coagulation test, was a sufficient evidence for the absence of LAC. Finally, a negative VDRL further supported the absence of antiphospholipid antibodies,

5. Oral anticoagulant or heparin treatment. Plasma with hemolysis were also discarded.

9 cc blood was collected without causing venous stasis, into vacutainer tubes containing 0.109 M trisodium citrate. Following centrifugation at 2000 rpm for 15 minutes, the plasma samples were stored in -80°C, as 1.5 cc aliquotes.

aPCR measurements were taken, using a modified aPCR kit (Instrumentation Laboratory Company, No: 200087). After diluting the plasma samples with factor V "reagent" plasma, we measured coagulation time, in the presence and absence of aPC. The results were given as aPC sensitivity ratio. The normal ratio is between 2.2 and 3.5. The results obtained by this method, were consistent with factor V Leiden mutation (sensitivity 99%). The statistical evaluation was made using student t test and Mann-Whitney U test and $p< 0.05$ was accepted as significant.

RESULTS

aPCR was found to be significantly higher in patients with BD (2.05 ± 0.21) as compared to the healthy controls (3.06 ± 0.19) ($p= 0.02$) (Figure 1). When we studied Behçet's patients with and without thrombosis separately, the BD group with aPCR positivity in thrombosis (+) compared with the healthy controls was significantly higher ($p= 0.01$). There was no significant difference between BD patients with and without thrombosis ($p= 0.4$) (Figure 2). If we consider BD patients as a whole group, there was also no significant difference in aPCR positivity with respect to sex and age (younger versus older than 40 years-old). The aPCR results of the patients and the healthy control groups are shown in Table 2.

DISCUSSION

Table 1. Demographic features of Behçet's patients and healthy controls

	Behçet's patients	Controls
Total	32	9
Male	20	6
Female	12	3
Age	35.4 (range: 20-56)	28.3
Venous thrombosis (+)	18	0
Venous thrombosis (-)	14	9

Table 2. aPCR results of patients and control groups.

		aPCR (+)		
	n	n	%	aPC ratio
Control group	9	0	0	3.06 ± 0.19
Behçet's patients	32	11	34.3	2.05 ± 0.21
Thrombosis (+)	18	8	44.4	1.89 ± 0.29
Thrombosis (-)	14	3	22.2	2.26 ± 0.31

The prevalance of BD differs in different populations and clinical findings of BD also show geographical and ethnic differences[6]. Although the main reason for arterial and venous thrombotic development in BD is endothelial damage, the contribution of genetic and enviromental factors are also well known. aPCR positivity has been reported in 5-13% of healthy population and nearly half of the patients with idiopathic venous thrombosis. Thrombosis is seen in nearly 25% of patients with BD and the role of aPCR has not been extensively studied in Behçet's patients with thrombosis. Gül et al. investigated the presence of factor V Leiden mutation causing aPCR in 64 BD patients. They showed that it was present among 38% of 32 patients with deep vein thrombosis compared to 9% of 32 patients without this complication. In this study aPCR positivity was shown in 10% of healthy controls[8]. Factor V Leiden mutation could not be detected in 12 of Behçet's patients with thrombosis. This may be explained by acquired aPCR. Mammo et al. showed the presence of the Factor V Leiden mutation in 37% of 8 patients with thrombosis compared with none of 15 patients without thrombosis[9]. Öner et al. demonstrated the presence of Factor V Leiden mutation in 60% of 5 patients with thrombosis, 18% of 39 patients without thrombosis and 7% of healthy controls[10]. Finally, Soy et al. found the presence of Factor V Leiden mutation in 42% of 12 patients with thrombosis compared with 11% of 18 patients without thrombosis and in 9% of healthy controls[11]. If we summarize the results of these four studies, the frequency of the factor V Leiden mutation in BD with and without thrombosis, ranges between 37.5-60% and 0-17.5%, respectively.

There are Behçet's patients with thrombosis, who do not have this mutation. Besides factor V Leiden mutation, pregnancy, oral contraceptives and antiphospholipid antibodies may also cause aPCR. In order to detect such cases of acquired aPCR, coagulometric tests should be preferred. It should be noted that all the previous studies mentioned above, have used only factor V Leiden mutation analysis for diagnosing aPCR. Our study is remarkable with using only coagulometric tests for diagnosing aPCR and the results of this study are very close to these previous studies in literature. Ko¾ar et al. showed increased incidence of aPCR

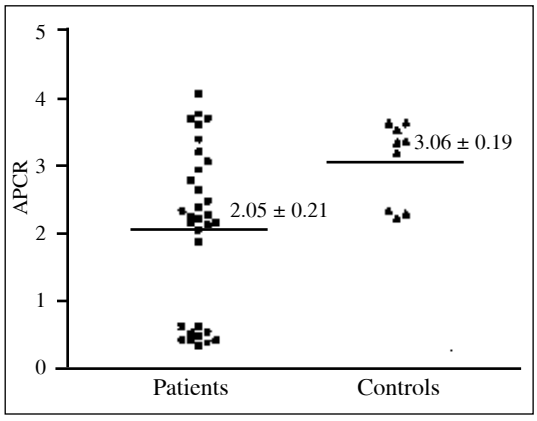

Figure 1. aPCR ratios of patient and healthy control groups (Mean ± SEM) p= 0.02.

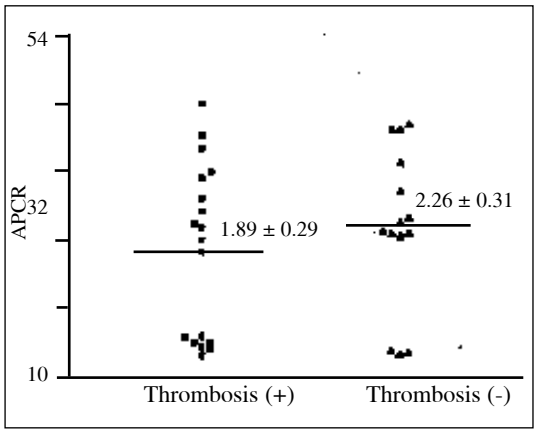

Figure 2. aPCR ratios of patients with BD with and without thrombosis (Mean ± SEM) p= 0.4.

(29.3%) in BD. In this study the prevalence of thrombosis was also found to be significantly increased (35%) in BD with aPCR[12].

In conclusion, aPCR frequency in BD detected by coagulometric tests as in this study is consistent with aPCR frequencies detected by Factor V Leiden mutation investigation in previous studies. aPCR frequency in Behçet's patients with thrombosis is not significantly different than other patients with idiopathic thrombosis. Presence of aPCR clearly increases the risk of thrombosis in BD. Hence, detection of aPCR in BD might be speculated as a possible indication for prophylactic anticoagulant treatment. Obviously, in further studies, aPCR should be investigated by performing both factor V Leiden mutation analysis and coagulometric tests concurrently. This would probably clarify the role of acquired aPCR in Behçet's disease.

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