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Survey of Factor V Leiden and Prothrombin Gene Mutations in Systemic Lupus Erythematosus

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Abstract: The two most common hereditary risk factors for thrombosis are factor V Leiden mutation and a prothrombin gene mutation. There is indeed a thrombotic tendency in patients with systemic lupus erythematosis (SLE) and it is not always associated with antiphospholipid antibodies. We aimed to determine the relationship between both factor V Leiden and prothrombin gene mutations and SLE. Using polymerase chain reaction (PCR) the factor V Leiden and prothrombin gene mutations were evaluated in 55 patients (20 children and 35 adults) with SLE. Although seven patients were found to have factor V Leiden mutation in the heterozygous state, two had the heterozygous G→A (20210) prothrombin gene mutation. Although one had these two mutations concurrently, these two patients did not have thrombosis. The factor V Leiden mutation frequency (12.7%) was higher than that of our general population (7.1%). On the other hand, seven of the patients with SLE had a thrombotic event. Although of these seven, four (57%) had factor V Leiden mutation, three (43%) had no mutation. Of 48 patients with no thrombotic history, only three had the factor V mutation (6.25%). The prevalence of the factor V Leiden mutation in SLE patients with and without thrombosis was significantly different by Fisher's exact test ($p < 0.05$). The risk of venous thrombosis in patients with factor V Leiden increased threefold compared to that in those without factor V Leiden mutation (odds ratio 20.1; CI 2.99–133.6). Although factor V Leiden mutation seems to play a role in the development of venous thrombosis in SLE, the development of thrombosis in SLE is multifactorial.

Keywords: Factor V Leiden; Gene mutation; Prothrombin; SLE; Systemic lupus erythematosus

Introduction

SLE is a multisystem disease which is the result of a complex series of inflammatory and immunological processes. Thromboembolic complications are frequently observed in SLE patients, particularly in the setting of active lupus with vasculitis and with antiphospholipid antibodies [1]. Nevertheless, hereditary risk factors for thrombosis may involve the process as well. The factor V Leiden mutation (G→A, position 1691) is the most common genetic disorder associated with an increased risk of thrombosis [2,3]. A similar point mutation (G→A change position 20210) in the 3′ untranslated region of the prothrombin gene is also known to be a novel risk factor. A threefold increased risk of venous thrombosis is a consequence of elevated plasma prothrombin levels [4,5]. In healthy Turkish individuals the prevalence of heterozygous factor V Leiden mutation and prothrombin gene mutation was found to be 7.1% and 2.2–2.7%, respectively [6–8].

We evaluated the contribution of these two mutations to the development of thrombosis in patients with SLE.

Patients and Methods

We enrolled a total of 55 unrelated patients with SLE (47 female, eight male) who fulfilled at least four criteria of

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the American College of Rheumatology for the classification of SLE into our study. This group included 20 children and 35 adults (mean age 27.9 ± 12.9; range 12–60 years). The mean disease duration was 6.07 ± 4.32 (0.8–23 years). We sampled our patients consecutively among those already under follow-up. Sampling for DNA analysis was performed when the patient was seen at the outpatient clinic or on admission to the ward. Extensive retrospective analysis of clinical charts regarding the presence of thrombosis was done before the factor V Leiden and prothrombin gene mutation results were known. Only objectively verified thrombotic events were included. All the paediatric patients and the majority of the adults were quiescent during the sampling. Seven patients (12.7%) had venous thrombosis, and all of them had active disease at the time. Each patient was investigated for the procoagulant G→A 1691 mutation in the factor V gene and the G→A 20210 mutation in the prothrombin gene. Each patient was also tested for IgG and IgM anticardiolipin antibodies by ELISA, and the results are expressed in GPL and MPL units. Levels higher than 10 GPL or MPL units are considered positive. The cut-off values were calculated as GPL and MPL levels in normal controls ±2SD.

DNA analysis

Polymerase chain reaction (PCR) was used to detect both the G→A 1691 mutation in exon 10 of the factor V gene and the G→A 20210 mutation in the 3′-untranslated region of the prothrombin gene. Briefly, digestion of amplified factor V DNA with *Mnl*I yielded a 267 base pair cleavage product that could be visualised on ethidium bromide-stained agarose gels, as previously described [9]. The G→A 20210 mutation in the 3′-untranslated region of the prothrombin gene was demonstrated by using a forward consensus primer and two reverse primers, one of which was specific for the wild type and the other for the mutated alleles. Allele-specific PCR products were visualised on ethidium bromide-stained agarose gels, as previously described [10]. A second amplification was performed to confirm the presence of each mutation.

Statistical Analysis

We used Fisher's exact test and calculated the odds ratio for estimation of thrombosis risk in the presence of procoagulant mutations. Data were analysed using SPSS+PC, and results are given as mean ± standard error. P values < 0.05 were considered significant.

Results

In our study group seven of 55 patients (12.7%) with SLE had a thrombotic event. Of these seven patients four (57%) had factor V Leiden mutation and none were found to carry the prothrombin gene mutation. Among the mutation-positive patients three had deep venous thrombosis of the legs, and one had pulmonary emboli. The remaining three mutation-negative patients also had deep venous thrombosis of the legs.

In the thrombosis-negative group the factor V Leiden mutation was present in three of 48 patients (6.25%), whereas prothrombin gene mutation was present in two of 48 patients (4.2%) (Table 1). It is interesting to note that one of these two prothrombin-positive patients is also a carrier of the factor V Leiden mutation.

Anticardiolipin antibodies were positive in 38% of the patients but in only two with thrombosis.

In our patients with SLE, the mutation frequency (12.7 %) was higher than that of the previously published normal Turkish population frequency (7.1%) [6,7,11], and was significantly higher in SLE patients with than without thrombosis ($p < 0.05$). Furthermore, the factor V gene mutation in the heterozygous state was found to be associated with at least a threefold increase in the risk of thrombosis (odds ratio 20.1; CI 2.99–133.6). With the exception of the presence of mutation, correlation analysis failed to show any significant effect of gender, disease duration or the presence of antiphospholipid antibodies on the risk for thrombosis.

Discussion

Activated protein C (APC) inhibits clothing by cleavage of factors Va and VIIIa into their inactive forms. However, as a result of a guanine to adenine point mutation at nucleotide 1691, mutated factor V is resistant to APC. Subjects heterozygous for the factor

Table 1. Prevalence of the factor V Leiden and prothrombin gene G20210A mutations in SLE patients with (T+) and without (T–) deep vein thrombosis

		Factor V Leiden	(%)	Prothrombin gene mutation (%)	
SLE	*n*	G,A*	G,G	G,A**	G,G
T+	7	4(57%)a	3 (43%)		7 (100%)
T	48	3(6.25%)b	45 (93.75%)	2(4.2%)	46 (95.8%)

*G,A heterozygous for the factor V gene G1691A mutation (factor V Leiden)
**G,A heterozygous for the prothrombin G20210A mutation
G,G no mutation.
a vs b, $p < 0.05$

V mutation show a 5–10-fold increased risk of thrombosis, whereas homozygous subjects show a thrombotic risk increased by 50–100-fold [12]. The second most common hereditary procoagulation factor is a G→A 20210 mutation in the 3′-untranslated region of the prothrombin gene, which results in elevation of plasma prothrombin levels and a threefold increased risk of venous thrombosis [4,5]. Thromboembolic events are reported in 25%–28% of patients with SLE [1].

However, in our study the frequency of thrombosis was less than that previously reported, perhaps because one-third of our patients were children. There are studies reporting a low prevalence of factor V Leiden in SLE patients [13,14]. Although in our study the frequency of factor V mutation was higher in patients with SLE with thrombosis, these patients were few in number. More SLE patients with thrombosis should be evaluated to address the role of factor V Leiden mutation in the development of thrombosis in SLE. Multivariate analysis showed that anticardiolipin antibody was not a risk factor for thrombosis in our SLE population, which is in agreement with a recent report [15]. Based on these findings, we think the additional risk factors may be required for manifestation of thrombosis in SLE.

Our study shows that factor V Leiden mutation may play an important role in development of venous thrombosis in SLE. However, molecular determinants of thrombosis in SLE seem to be as multifactorial as the nature of the disease.

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