PREVALENCE OF FACTOR XIII VAL34LEU POLYMORPHISM IN THROMBOSIS CASES IN THE SOUTHEAST OF TURKEY AND ITS CORRELATION WITH THROMBOSIS


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ABSTRACT

Background: Thrombosis is triggered by a shift in the balance of procoagulant and anticoagulant factors due to acquired or inherited causes. Factor XIII plays an important role in the stabilization of the linkage between fibrins. Three different genetic structure of Valine34Leucine polymorphism in FXIIIA have been described. Valine/Valine structure has been identified as the homozygous normal (wild-type), whereas Valine/Leucine and Leucine/Leucine structures have been identified as heterozygous and homozygous mutants respectively. Genetic polymorphisms in FXIII-A subunit vary substantially from society to society. The primary objective of this study was to determine the relationship between factor XIII polymorphisms and arterial/venous thromboembolism in the southeast of Turkey.

Methods: A total of 127 patients with arterial and venous thrombosis were included as the study group and 102 healthy subjects with no thromboembolic disorders were included as the control group. Val34Leu polymorphism in FXIII was investigated in both groups using PCR (Polymerase chain reaction).

Results: The prevalence of the polymorphism in the study and control groups were compared with chi-square test, no statistically significant differences were found (the prevalence in the study and control group are 68.5% and 66.7% for V/V allele, 29.2% and 29.4% for V/L allele, and 2.4% and 3.9% in L/L allele respectively, with p = 0.787). When deep vein thrombosis (DVT) diagnosed female patients group (n = 8) was compared to the healthy female control group, V/L allele was significantly lower, whereas L/L allele was significantly higher (p = <0.01).

Conclusion: This study indicates that there is no evidence for association between factor XIII-A Val34Leu polymorphism and arterial/venous thromboembolism. The significant result we found for the DVT patients should be strengthened by further studies with greater number of cases. In future, further studies are needed with more specific groups and with greater numbers of patients.

Keywords: Factor XIII, polymorphism, thrombophilia, venous-arterial thrombosis.

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Introduction

Factor XIII (Fibrin-stabilizing factor) is a tetrameric structural pro-transglutaminase from the transglutaminase family. Factor XIII activity is associated with the structure of fibrin clot. Factor XIII turns into XIII-A, which is an active transglutaminase, in the result phase of coagulation cascade together with Ca++ and thrombin activation. The main aim of Factor XIII is to connect ε(δ-glutamyl) lysyl-bound fibrin with δ- fibrin, α-chains and α2-plasmin inhibitor with the help of non-covalent bonds. In this way, it improves the mechanical resistance of the clot and increases its resistance against fibrinolysis\(^{(1,3)}\).

Factor XIII-A is the form of Factor XIII which carries risk in arterial or venous thromboembolisms. 5 different gene polymorphisms associated with Factor XIII-A have been defined so far. The most common one is polymorphism (FXIII-A
subunit Val34Leu\(^{(3, 4)}\), which occurs when leucine replaces valine in the 34\(^{th}\) position of Factor XIII-A gene. The clots that are formed in Factor XIII Val34Leu gene polymorphism are thin and less porous. In the prothrombin concentrations that are effective in clot structure, the thin clots are very resistant against fibrinolysis and associated with increased risk of thrombosis\(^{(5)}\). Previous studies have shown different results regarding Factor XIII Val34Leu polymorphism’s protective, risk factor or ineffective status in its correlation with arterial and venous thrombosis\(^{(6-10)}\).

In current study, we evaluated the prevalence of Factor XIII Val34Leu polymorphism in the thrombosis cases in the Southeastern Anatolia of Turkey and its correlation with thrombosis.

**Material and methods**

This study was conducted at the Dicle University School of Medicine between December 2010 and May 2012. All analyses were performed in accordance with the principles of the Declaration of Helsinki. 127 patients (65 males / 62 females) with arterial and venous thrombosis, who were monitored in internal medicine, neurology, cardiology, chest diseases, cardiovascular surgery and general surgery clinics, and 102 healthy people without thromboembolism, were involved in this study.

The diagnoses of the participant patients were conducted through imaging methods. The patients with cerebral infarction and sinus vein thrombosis were examined by computed tomography (CT), magnetic resonance imaging (MRI), CT angiography and MRI angiography; the patients with deep vein thrombosis by Doppler ultrasonography; the patients with pulmonary embolism by ventilation-perfusion scintigraphy, thorax tomography and CT pulmonary angiography; the patients with acute coronary syndrome by coronary angiography and finally the patients with portal, hepatic, splenic and mesenteric artery-vein thrombosis were examined by portal hepatic Doppler ultrasonography, upper abdominal tomography and MRI.

The patients were divided into two groups as those with arterial and venous thrombosis. Acute myocardial infarction, cerebral infarction and superior mesenteric artery thrombosis were taken into the group with arterial thrombosis. The venous thrombosis group was divided into two groups as the typically located, in the form of deep vein thrombosis and pulmonary arterial thrombosis, and atypically located in the form of portal vein thrombosis, splenic vein thrombosis, hepatic vein thrombosis, vena cava thrombosis, upper extremity thrombosis, retinal vascular thrombosis and sinus vein thrombosis. Splenic vein thrombosis, hepatic vein thrombosis and vena cava thrombosis were examined within portal vein thrombosis.

2 ml venous blood sample was taken into EDTA tubes in order to detect Factor XIII V34L gene polymorphism. DNA isolation was performed on blood sample in the EDTA tube in Roche-Magna Pure Compact automated DNA isolation device with ready isolation kit. The isolated DNA sample was added to the master mix solution, which was prepared in capillary system devices for the detection of polymorphism. 7.6 μl of water, PCR grade solution, 1.6 μl MgCl2, 4.0 μl.1 agent musk (specific parameters agents including primers and probes), and 2.0 μl. of enzyme mix was used during the preparation of the solution. After 5 L of DNA sample was added to 15 ~ master mix mixture, centrifugation was performed for capillary to drop to the bottom of the tube. The results were conducted in Light Cycler 2.0 instrument and evaluated, and amplification values belonging to Factor XIII V34L genotype were gathered. The presence of mutant (34L) was fixed at 58.8 C melting point (Tm) while wild-type (normal allele) presence (V34) was fixed at 66,3 C.

**Statistical analyses**

Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Independent variables were compared through student t test. Chi-square test was employed to detect dependent variables. The average value of the data was shown as ± SD. The results were accepted at 95% confidence interval and p <0.05 standard deviation level.

**Results**

127 patients (aged 38.43±12.47) who were monitored due to thrombosis and 102 healthy people (aged 35.35±13.15) without thromboembolism were included in the control group. When the participant patients were compared with the control group, there were no significant differences in terms of age (Table 1). The distribution of the patients in line with the diagnoses is as follows: 24.4% cerebral infarction (ischemic stroke, IS), 22.4% acute myocardial infarction (MI), 17.32% cerebral sinus venous thrombosis (CSVT), 11.81%
pulmonary thromboembolism (PTE), 11.02% deep vein thrombosis (DVT), 9.9% portal vein thrombosis and 3.9% the others consisting of upper extremity thrombosis, superior mesenteric artery thrombosis and retinal vein thrombosis.

The patients with thrombosis were categorized as arterial and venous. These groups were compared with the control group in terms of gene polymorphism and no statistically significant difference was found (p: 0.624).

Discussion

Factor XIII Val34Leu polymorphism has important ethnic heterogeneity. While leucine allele frequency is quite high in Western countries, this ratio decreases in Eastern countries. While leucine allele frequency is 48-51% in England, it is 45,8% in the USA, 43% in Italy, 45,1% in Hungary, 50,2% in France and 28,9% in Brazil but it is very rare in Japan in that the ratio is about 2,5% (6, 8, 17, 20, 21).

In our study, Factor XIII 34 V/V Wild homozygous mutant normal type was fixed at 68,5%, Factor XIII 34 V/L heterozygous type was fixed at 29,2%, and homozygous mutant type Factor XIII 34 L/L polymorphism was fixed at 2,4% in the participant patients.

In our country, in the study conducted by Diz-Küçükkaya et al., the frequency of gene polymorphism was found to be FXIII 34 V/V 62,6%, FXIII 34 V/L 35,7%, FXIII 34 L/L 1,6% in the participant patients with thrombosis who were diagnosed with antiphospholipid syndrome and the patients with abortions whose anti phospholipid antibody was positive and/or the patients with thrombocytopenia, all of whom were 82 in total (22).

In our study, in 102 healthy people in the control group, polymorphism rates were found to be FXIII 34 V/V 66,7%, FXIII 34 V/L 29,4%, FXIII 34 L/L 3,9%. Similar results were gathered in the study conducted by Diz-Küçükkaya.

In the study conducted by Kohler et al., 398 patients with coronary artery disease and 198 clinically healthy people were involved in the study. The prevalence of mutations was found to be lower in those diagnosed with coronary artery disease and had MI compared to those who were ill but did not have MI (p=<0.005). According to these results, G → T mutation encoding FXIII V34L was evaluated as a protective against MI(6). Also, in the study conducted by Gemmati et al., the patients with coronary artery disease, IS and intracerebral haemorrhage were examined and eventually it was found that while FXIII 34 V/L polymorphism had a protective effect against MI and IS, it was found to be a risk factor in terms of intracerebral haemorrhage(8).

Table 1: Demographic characteristics of the patient and control group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patient N:127</th>
<th>Control N:102</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>38,43±12,47</td>
<td>35,35±13,15</td>
<td>0,071</td>
</tr>
<tr>
<td>Gender ( M/F )</td>
<td>65/62</td>
<td>42/60</td>
<td>0,098</td>
</tr>
</tbody>
</table>

Table 2: Factor XIII polymorphisms of the patients in the study and control group.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor XIII 34 V/V Wild Type Homozygotes</td>
<td>87 (68,5%)</td>
<td>68 (66,7%)</td>
</tr>
<tr>
<td>Factor XIII 34 V/L Homozygotes</td>
<td>37 (29,2%)</td>
<td>30 (29,4%)</td>
</tr>
<tr>
<td>Factor XIII 34 L/L Mutant Homozygous</td>
<td>3 (2,4%)</td>
<td>4 (3,9%)</td>
</tr>
</tbody>
</table>

Table 3: Distribution of the diagnoses of patients according to Factor XIII gene polymorphism.


<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patient N:127</th>
<th>Control N:102</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMI</td>
<td>16 (57,1%)</td>
<td>11 (39,3%)</td>
<td>1 (3,6%)</td>
</tr>
<tr>
<td>IS</td>
<td>23 (74,2%)</td>
<td>8 (25,8%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>CSVT</td>
<td>17 (77,3%)</td>
<td>5 (23,7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>PTE</td>
<td>10 (66,7%)</td>
<td>5 (33,3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>PVT</td>
<td>7 (58,3%)</td>
<td>5 (41,7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>DVT</td>
<td>10 (71,4%)</td>
<td>2 (14,3%)</td>
<td>2 (14,3%)</td>
</tr>
<tr>
<td>Others</td>
<td>4 (80%)</td>
<td>1 (20%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Table 4: Parameters
In some of the previous studies, FXIII polymorphism was found to decrease and/or be protective against MI risk in the ones with major cardiovascular risk\(^{(16,23)}\). In the study conducted by Roldan et al. on Mediterranean population with the patients under 45 and undergoing MI, FXIII 34 V/L polymorphism was not found to have a protective effect and even homozygous mutant allele was found to be significantly higher in the people under 35 (9). 28 of the participant patients in our study were diagnosed with MI.

FXIII V34L gene polymorphism distribution of the patients were V/V 57,1%, V/L 39,3%, L/L 3,6%. When these were compared with the control group, no statistically significant results were obtained. No interaction was found between FXIII gene polymorphism and MI development in our study.

Shemirani et al. examined 496 patients with ischemic stroke and control group with 1146 people and another group chosen from the control group according to age and gender in terms of FXIII 34 V/L in their study. In this study, homozygous or heterozygous variants of Leu34 allele were found to be protective\(^{(12)}\). In the study conducted by Elbaz et al., Leu allele was found to be statistically significantly lower in the patient group compared to the control group. Although how FXIII gene mutation made such an impact is not known, they finally found that FXIII V34L polymorphism was negatively correlated with IS and that Leu alleles had a protective effect on cerebral artery occlusion\(^{(13)}\).

In the meta-analysis study conducted by Li B et al. including 3807 cases and the control group with 4993 people, the correlation between FXIII V34L polymorphism and IS was examined. When the results of 16 studies were gathered and examined, no correlation was obtained between FXIII V34L polymorphism and IS\(^{(24)}\). There were 31 patients diagnosed with IS in our study and no statistically significant results were obtained when compared to the control group.

In VTE etiology there have been various results in different studies regarding the status of FXIII 34 V/L gene polymorphism. In the case-control study by Van Hylckama Vlieg et al., the effect of FXIII 34 V/L polymorphism, FXIII activity, and subunit levels were examined in terms of thrombotic risk in DVT. High levels of FXIII were correlated with the possibility of lower thrombotic risk\(^{(15)}\).

In another case-control study by Catto et al., 221 patients with VTE diagnosis were examined. Eventually FXIII 34 V/L polymorphism was found to be protective against DVT\(^{(19)}\). Again in another case-control study, Zidane et al. examined the frequency of TAFI-438G/A and FXIII 34 V/L polymorphisms in the patients with proven diagnosis of pulmonary thromboembolism. Consequently, they reported that FXIII 34 V/L polymorphism could be protective against venous thromboembolism\(^{(29)}\).

In the meta-analysis study conducted by Wells et al. including 3196 VTE patients and 4909 healthy individuals, they found that both homozygous and heterozygous FXIII gene polymorphisms were protective against VTE and reported that such effects were more in the homozygous variant\(^{(30)}\). As to our study, there were 14 patients with DVT diagnosis. FXIII polymorphism distributions of the patients were V/V 71,4%, V/L 14,3% and L/L 14,3%. There were no statistically significant results when compared to the control group. While the genotype distribution of 15 PTE cases were V/V 66,7%, V/L 33,3%; L/L polymorphism was not fixed and no significant results were gathered when statistical comparisons were made. In the ones diagnosed with PTE, frequency of FXIII V34L polymorphism was not different from that of control group. Ultimately, no correlation was found between venous thromboembolism and FXIII gene mutation.

In conclusion, no consensus has been achieved regarding the correlation between FXIII 34 V/L gene polymorphism and arterial and venous thrombosis. In our study, no results have been obtained as to whether this mutation increases the predisposition to thrombosis or plays a protective role in the patients with thrombosis. More case studies are needed to arrive at a final decision.

References


