Venous thromboembolism (VTE) refers to the development of thrombi into the veins and an occlusion in the pulmonary artery and branches caused by embolic fragments from these thrombi.\(^1\) Since in general deep vein thrombosis (DVT) occurring with the formation of thrombi in deep veins of lower extremity and its complication pulmonary embolism (PE) are associated, venous thromboembolism (VTE) term is often used for DVT and/or PE. VTE is an

**Objectives:** To evaluate patients diagnosed with venous thromboembolism (VTE) in terms of hereditary thrombophilic risk factors and to assess genetic and biochemical factors affecting the development of VTE.

**Methods:** Sixty patients with VTE and 23 control subjects without VTE were retrospectively evaluated. Prevalence of thrombophilic risk factors and parameters like demographic data, clinical follow-up duration were examined with genetic, biochemical and radiological investigations.

**Results:** Mutations were detected in the genes of Factor V Leiden in 37.4%, Factor II in 13.4%, Methylenetetrahydrofolate reductase C677 in 47.5%, Methylenetetrahydrofolate reductase A1298C in 53.3%, Plasminogen activator inhibitor-1 in 31.6%, Angiotensin converting enzyme in 39.0%, and Factor V H1299R in 8.3% of patients. Protein C deficiency was detected in 25 patients (41.7%), free Protein S deficiency was detected in 25 patients (41.7%) in study group and 3 subjects (13.0%) in control group, and this difference was statistically significant (p=0.023). Antithrombin III deficiency was detected in 1 patient (1.7%) in study group. Elevated homocysteine was higher in study group compared to controls, and the difference was statistically significant (p=0.02).

**Conclusion:** Determination of hereditary risk factors in VTE patients will provide family members who have hereditary risk factors, but did not suffer attack to be protected against thromboembolic attacks by taking simple measures against acquired factors.

**Keywords:** Hereditary thrombophilia, superficial thrombophlebitis, venous thromboembolism
important cause of morbidity and mortality. Many factors play a role in the etiology of VTE. In its pathogenesis, the triad consisting of stasis, vascular wall injury (endothelial damage) and hypercoagulability, which was described by Virchow is still valid. Hemostasis diseases causing thrombosis are named as thrombophilia. There are hereditary and acquired causes in the etiology of thrombophilia. Hereditary factors predispose to VTE, begin in a young age and show a tendency to repeat. Therefore, screening of hereditary factors in VTE patients is crucial for treatment and follow up plan.

The objective of this study was to retrospectively determine the prevalence of thrombophilic factors such as VG 1691A [Factor V Leiden (FVL)], Factor V H1299R, Prothrombin G20210A (Factor II), Methylene tetrahydrofolate reductase C677T (MTHFR C677T), methylenetetrahydrofolate reductase A1298C (MTHFR A1298C), plasminogen activator inhibitor-1 (PAI-1), angiotensin converting enzyme (ACE) gene mutations with protein C (PC), free protein S (PS), antithrombin III (AT III) and homocysteine in patients with VTE, and to evaluate the effects of these factors and parameters such as patients' demographic data, clinical and radiological findings, development patterns, pulmonary embolism and cancer association on treatment plan and patient follow up.

Methods

Study Population and Design

The study was approved by the institutional ethics committee (approval date: December 22, 2011 and decision number: 2011/88). Patients were informed about the study, and their written consents were obtained. Between January 2004 and December 2012, a total of 60 patients who had hereditary thrombophilic risk factors and were treated and followed in our institution due to the diagnosis of VTE, and 23 patients who were not developed VTE but diagnosed with venous insufficiency were included in this study. Genetic, biochemical and radiological investigations were examined from the patient files and the data obtained were recorded. Patients' demographic data such as age and gender, physical examination findings, VTE development patterns, PE development, cancer and recurrence association, comorbidities, lower extremity venous Doppler USG results, AT III, PC, PS, homocysteine analysis outcomes, thrombophilia panel (FVL, Factor V H1299R, Factor II, MTHFR C677T, MTHFR A1298C, PAI-1 and ACE mutation outcomes), follow up durations and recovery time on USG were obtained from the hospital records and analyzed. Patients with missing data were excluded from the study.

Genetic Analysis

Peripheral venous blood samples were put into the standard sterile tubes and kept at -20°C. DNA analysis was performed in the peripheral blood samples using Invisorb Sp in Stool DNA Kit (Invitek, Berlin, Germany). The presence of DNA was viewed in agarose gel. Mutations were studied with DNA Light Cycler PCR. FVL, Factor II, MTHFR C677T, MTHFR A1298C, PAI-1 and ACE mutations were studied. LightCycler Hybridization Probe Kit was used to investigate the polymorphisms of ACE, PAI-1, MTHFRC677T, MTHFR A1298C, FVL and Factor II genes.

Biochemical Analysis

The samples were put into citrate containing vacuum laboratory tubes to investigate PC, free PS and AT III. The parameters were determined in the plasma obtained from the standard centrifuge process with chromogenic method. Stago STA Compact (Diagnostica Stago S.A.S., France) full automated coagulation system was used for the analysis. The samples were taken into the vacuumed serum separation tubes. The measurements were made in the serum obtained after the standard centrifuge process. When data obtained from the measurement were processed, normal range was accepted as 70-140% for PC, 60-140% for free PS, 80-120% for AT III and 5-12 µmol/mL for homocysteine.

Radiological Analysis

In this study, examination of common femoral, deep and superficial femoral, popliteal vein, saphena and crural branches with doppler USG of both lower extremities was performed with Siemens Sonoline Antares Ultrasonography device. Dilatation of vein diameter, absence of response to compression with the probe, absence of filling (flow) with color doppler were accepted as positive findings for DVT by the radiologists. Vein diameter returning to normal, response received to compression, and flow observed with color doppler were accepted as recovery on doppler.

Statistical Analysis

Statistical analysis of the data was performed using SPSS (Statistical Package for Social Sciences) for Windows version 15.0 package software. In the comparison of subgroups, continuous variables are expressed as mean±standard deviation, and categorical variables as % (percentage). Parametric variables were evaluated with t test, and One-way ANOVA test, non-parametric variables with Chi-square test, Kruskal-Wallis test and Mann-Whitney U test, multiple comparisons with Post Hoc tests (Scheffe, Games Howell), and factorial effects with factor analysis tests. P<0.05 values were considered statistically significant.
## Results

The mean ages were 50.9±16.4 and 47.4±14.6 years in the study and control groups, respectively. Of the patients in the study group, 35 (58.3%) were female and 25 (41.7%) male in the study group, while 15 (65.2%) of the control subjects were female and 8 (34.8%) were male in the control group. There were no statistically significant differences between the groups in terms of age (p=0.566) and gender (p=0.373). The patterns of VTE and superficial thrombophlebitis (ST) development in the study and control groups are given in Table 1.

Percentage distribution of VTE development patterns in the study group and percentage distribution of ST development patterns in the control group are given in Figure 1 and 2, respectively.

The development of VTE was spontaneous in majority of the study group (40.0%) and related to bed treatment (36.7%), while superficial thrombophlebitis (ST) was due to other rare causes (21.7%) in the control group. In the study group, number of female patients (45.7%) with VTE related to bed treatment was higher than male patients (24.0%). This difference was statistically significant (p=0.020). In the study group, development of VTE was associated with a reason in 24 (68.6%) female and 12 (48.0%) male patients, VTE was developed in 11 (31.4%) female and 13 (52.0%) of male patients.

VTE was accompanied by ST in 10 cases (16.7%), while DVT and ST association, namely thrombi both in deep veins and superficial veins of the lower extremity was seen in 9 (15.0%) of these patients. The remaining one

### Table 1. Distribution of VTE and ST development patterns according to the groups and gender

<table>
<thead>
<tr>
<th></th>
<th>Spontaneous</th>
<th>Bed treatment</th>
<th>Long journey</th>
<th>Pregnancy</th>
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<tr>
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<td>6</td>
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<td>4</td>
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</tr>
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<td><strong>Study group</strong></td>
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<td></td>
</tr>
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<td>2</td>
<td>5.7</td>
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<td>6</td>
<td>24.0</td>
<td>5</td>
<td>20.0</td>
</tr>
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<td>22</td>
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<td>7</td>
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<td>19</td>
<td>38.0</td>
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<td>34.9</td>
<td>28</td>
<td>33.7</td>
<td>11</td>
<td>13.3</td>
</tr>
</tbody>
</table>

VTE: Venous thromboembolism; ST: Superficial thrombophlebitis.

### Figure 1. Venous thromboembolism (VTE) development patterns in the study group.

### Figure 2. Superficial thrombophlebitis (ST) development patterns in the control group.
In the control group, development of ST was not related to homocysteine in 18 (30.0%) patients. PS in 1 (1.7%), elevated AT III in 4 (6.7%) and elevated free PS deficiency in 5 (8.3%), elevated PC deficiency in 5 (8.3%), elevated PC in 2 (3.3%), and heterozygous Factor V H1299R mutation in 1 (1.7%) patient. Recurrent VTE was found in 3 (5%) patients.

Among the patients with spontaneously developed VTE; heterozygous FVL gene mutation was found in 5 (8.3%), heterozygous Factor II mutation in 1 (1.7%), homozygote MTHFR C677T mutation in 3 (5.0%), heterozygous MTHFR C677T mutation in 6 (10.0%), homozygote MTHFR A1298C mutation in 5 (8.3%), heterozygous MTHFR A1298C mutation in 9 (15.0%), homozygous PAI-1 mutation in 1 (1.7%), heterozygous PAI-1 mutation in 4 (6.7%), I/D (deletion) homozygote mutation in 7 (11.7%), and heterozygous Factor V H1299R mutation in 1 (1.7%) patient.

PC deficiency was found in 15 (25.0%), elevated PC in 4 (6.7%), free PS deficiency in 18 (30.0%), elevated AT III in 3 (5.0%), AT III deficiency in 1 (1.7%), and hyperhomocysteinemia (elevated homocysteine) in 11 (18.3%) patients.

In the study group, development of VTE was not related with bed treatment in 38 (63.3%) patients, while VTE was developed due to bed treatment in 16 (45.7%) female and 6 (224.0%) male patients. This difference between the female and male patients was statistically significant (p=0.020). VTE was accompanied by ST in 10 cases (16.7%), while ST was developed due to bed treatment in 17 (13.1%) patients. The mean recovery time was 16 months, and the duration of treatment and follow up was 20 months. In the patients who developed VTE due to long journey, cancer association and PE association were not observed, while recurrent VTE was found in 3 (5%) patients.

Among the patients who developed VTE due to pregnancy; homozygous FVL gene mutation was found in 3 (5.0%), heterozygous FVL mutation in 2 (3.3%), homozygous Factor II mutation in 1 (1.7%), heterozygous Factor II mutation in 2 (3.3%), homozygote MTHFR C677T mutation in 1 (1.7%) and heterozygous MTHFR C677T mutation in 1 (1.7%) and heterozygous MTHFR A1298C mutation in 2 (3.3%), homozygote MTHFR A1298C mutation in 2 (3.3%), heterozygous PAI-1 mutation in 1 (4.3%), I/D heterozygote ACE mutation in 1 (4.3%) and homozygote Factor V H1299R mutation in 1 (1.7%) patient. Recurrent VTE was found in 3 (5%) patients.

In the control group, development of ST was not related to long journey in 20 (86.9%), while ST was developed due to long journey in 3 (13.1%) patients. The mean recovery time was 13 months, and the duration of treatment and follow up was 10 months.

In the control group, homozygous FVL gene mutation was found in 1 (4.3%), heterozygous FVL mutation in 1 (4.3%), homozygote MTHFR C677T mutation in 1 (4.3%), homozygous MTHFR A1298C mutation in 2 (8.7%), heterozygous PAI-1 mutation in 1 (4.3%), I/D heterozygote ACE mutation in 1 (4.3%) and homozygote Factor V H1299R mutation in 1 (4.3%) patient. Recurrent VTE was found in 1 (4.3%) patient.

In the study group, development of VTE was not related to pregnancy in 54 (90.0%) patients, while VTE was developed due to pregnancy in 6 (10%) patients. The development of VTE was related to pregnancy in 6 (17.1%) female patients. The mean recovery time was 11 months, and the duration of treatment and follow up was 24 months.

Among the patients who developed VTE due to pregnancy, cancer association was seen in 1 (1.7%) patient, while PE association was found in 1 (1.7%) patient. Recurrent VTE was found in 1 (1.7%) patient.
Among the patients with VTE developed due to pregnancy, homozygous FVL gene mutation was found in 1 (1.7%), heterozygous FVL mutation in 3 (5.0%), heterozygous Factor II mutation in 1 (1.7%), heterozygous MTHFR C677T mutation in 3 (5.0%), heterozygous MTHFR A1298C in 4 (6.7%), homozygous PAI-1 mutation in 1 (1.7%), heterozygous PAI-1 mutation in 2 (3.3%), I/D heterozygote ACE mutation in 1 (1.7%), D/D (deletion) homozygous mutation in 2 (3.3%), and heterozygous Factor V H1299R mutation in 2 (3.3%) patients. PC deficiency was found in 1 (1.7%), free PS deficiency in 1 (1.7%), elevated AT III in 2 (3.3%), and elevated homocysteine in 1 (1.7%) patients.

In the control group, development of ST was not related to pregnancy in 20 (87.0%), while ST was developed due to pregnancy in 3 (20.0%) female patients. The mean recovery time was 8 months, and the duration of treatment and follow up was 12 months.

While among the patients in the control group who developed ST due to pregnancy polymorphism was not found in any gene in 1 (4.3%) patient, heterozygous MTHFR A1298C gene mutation was found in 1 (4.3%), heterozygous PAI-1 mutation in 1 (4.3%), I/I (insertion) homozygote ACE mutation in 1 (4.3%), and heterozygous Factor V H1299R mutation in 1 (4.3%) patient. Whereas biochemical outcomes were normal in 2 patients, elevated PC, free PS, and AT III values were found in 1 (4.3%) patient.

In the control group, development of ST due to other rare reasons was found in 2 (8.7%) patients.

When the patients were evaluated according to the extremity involvement, upper extremity involvement was not observed in any patient in the study group, while upper extremity was involved in only 2 (8.7%) patients in the control group. When evaluated according to the left or right side involvement, no significant difference was observed between the study and control groups. Distribution of the involved extremity is shown in Table 2.

Distribution of recurrence, PE development and cancer association according to the hereditary risk factors is given in Table 3.

Distribution of recurrence, PE development and cancer association according to the biochemical risk factors is given in Table 4.
Discussion

VTE is a disease occurring with multifactorial effect with involvement of numerous hereditary and acquired factors. Screening of hereditary thrombophilic factors in VTE patients is crucial for treatment and follow-up planning. [1] In our study, no any polymorphism was seen in 3.6% of the patients who developed VTE, while mutations were found in MTHFR A1298C gene in 53.3%, MTHFR C677T gene in 47.5%, ACE gene in 39.0%, FVL in 37.4%, PAI-1 in 31.6%, Factor II in 13.4%, and Factor V H1299R in 8.3% of the patients. In previous studies, polymorphism was found by 53.3% in MTHFR A1298C gene, 48.3% in MTHFR C677T gene, 38.3% in Factor V G1691A, 36.7% in ACE gene, 31.7% in PAI-1 gene, and 8.3% in Factor V H1299R gene. [5,6] Our results were consistent with the previous studies.

FVL mutation is a condition with autosomal dominant inheritance that is seen by 40% to 60% in patients with hereditary thromboembolism, and its prevalence varies between 2-15%. [7] In our study, FVL mutation was seen in 30.4% of the patients in the control group (26.1% heterozygous + 4.3% homozygote). In a study by Koster et al., [8] the risk for development of VTE was reported to be 7 folds higher in patients with FVL mutation, while Ridker et al. [9] found 3 times higher risk for the development of VTE. In our study, the presence of FVL mutation increased the risk for development of VTE by two folds. On the other hand, in our study FVL mutation was found by 31.7% in patients in patients with DVT alone, 5.0% in patients with DVT plus pulmonary embolism, and 1.7% in patients with pulmonary embolism alone. In a study by Caprini et al., [10] FVL mutation was found in 23% of all patients with DVT, and 9% of the patients with a history of pulmonary embolism. The prevalence of FVL was higher in the patients who developed spontaneous VTE compared to the patients who developed VTE due to long journey, and the difference was statistically significant (Scheffe p=0.004) (Games-Howell p=0.037).

The mechanism of hypercoagulability related to Factor II mutation is not known, but it was thought that this may be associated with the increased amount of thrombin. [11] In our study, Factor II mutation was found in 13.4% of the patients in the study group (11.7% heterozygous + 1.7% homozygote). In a study by Altıntaş et al. [12] on 52 patients with DVT, no homozygous mutation was found in Factor II gene, while heterozygous mutation was found by 11%. The incidence of heterozygous mutation in Factor II in that study was consistent with our study.

In our study, MTHFR A1298C mutation which had the highest polymorphism in the study and control group was seen in 53.3% of the patients in the study group (heterozygote 38.3% + homozygote 15.0%) and in 69.5% of the patients in the control group (heterozygote 56.5% + homozygote 13.0%). MTHFR C677T mutation was found in 47.5% of the patients in the study group (heterozygote 36.7% + homozygote 11.9%). However, MTHFR gene mutations are seen also in healthy individuals (The most common mutation is C677T followed by A1298C). Heterozygous mutations were seen in 37.8-51% and homozygous mutations in 5.4-17% of healthy individuals in Australia, North America, and Europe. [13] In our study, we found MTHFR C677T mutation in 39.1% (heterozygote 30.4% + homozygote 8.7%) and MTHFR A1298C in 69.5% (heterozygote 56.5% + homozygote 13.0%) in healthy individuals.

Table 4. Distribution of recurrence, pulmonary embolism development and cancer association according to biochemical risk factors

<table>
<thead>
<tr>
<th></th>
<th>Decreased PC</th>
<th>Decreased PS</th>
<th>Decreased AT III</th>
<th>Elevated homocysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrence</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Female</td>
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<td>4</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Male</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>7</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Pulmonary embolism development</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0</td>
<td>3</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Male</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
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<td>3</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Cancer association</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Male</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

PC: Protein C; PS: Protein S; AT: Antithrombin.
PAI-1 is a rapid inhibitor of tissue type plasminogen activator. In our study, PAI-1 mutation was found in 31.6% of the patients in the study group (heterozygote 23.3% + homozygote 8.3%) and in 39.1% of the patients in the control group (heterozygote 30.4% + homozygote 8.7%).

In a study by Philipp et al., investigating the relationship between ACE gene polymorphism and venous thrombosis in patients who underwent hip arthroplasty, it was concluded that the presence of D7D genotype increased the risk for venous thrombosis compared to the presence of I/D and I/I genotypes. In the same study, thrombotic events were 11.7 times higher in the patients with ACE D/D genotype compared to ACE I/I genotype, and 5 times higher in the patients with ACE D/I genotype compared to ACE I/I genotype. In our study, ACE mutation was found in 39.0% of the patients in the study group (homozygous deletion (D/D) 22.0% + heterozygote (I/D) 8.5% + homozygous insertion (I/I) 8.5%) and in 47.7% of the patients in the control group (homozygous deletion (D/D) 21.7% + heterozygote (I/D) 13.0% + homozygous insertion (I/I) 13.0%). In our study, no significant difference was found between the groups and genders in terms of ACE mutations. However, the incidence of ACE gene mutation was higher in the patients who developed VTE due to bed treatment compared to the patients who developed VTE related to long journey (Games-Howell p=0.038).

Factor V H1299R mutation was found in 8.3% of the patients in the study group (all heterozygote) and 13.0% of the patients in the control group (all heterozygote). When the study group was evaluated according to genders in terms of the presence of Factor V H1299R mutation, mutation was seen by 14.3% in female and 0.0% in male patients, and the difference was statistically significant (p=0.048). Although there are differences between ethnic groups in terms of Factor V H1299R mutation, its prevalence was reported as 9.5%-15% worldwide. However, its association with thromboembolism is lower than FVL mutation.

In the literature there are some reports revealing the association pregnancy-related venous thromboembolism and ischemic stroke with Factor V H1299R mutations. The incidence of PC deficiency is 1/16000-36000 in general population. Because PC deficiency is seen by 10% in patients under 40 years old who suffer VTE, and increases the risk for VTE by 6 folds, PC levels should be investigated in each young patient who develops VTE. In our study, no significant difference was found between the groups and gender in terms of PC values (p=0.05). PC deficiency was found in 26.1% of the subjects in the control group and 41.7% of the patients in the study group.

This study has some limitations. The number of patients was relatively low because of the exclusion of patients with missing records. In addition, patients were followed up by different physicians and the study was designed as a retrospective study. However, our results will be guiding for further studies to be conducted on this issue.

In conclusion, since venous thromboembolism is a systemic disease and a serious cause of morbidity and mortality, hereditary and acquired risk factors should be evaluated together, and family history and drug use should be meticulously questioned. Determination of hereditary risk factors in VTE patients, will provide family members who have hereditary risk factors, but yet did not suffer attack to be protected against thromboembolic attacks by taking simple measures against acquired factors. Hereditary risk factors for VTE should be evaluated individually for each patient in details, and treatment and follow up should be planned according to the results. Determination of person specific risk factors could be helpful in prediction of the prognosis and guiding for treatment and follow up. Further comprehensive prospective randomized studies are needed in order to support and confirm the results of this study.

Disclosures
Ethics Committee Approval: The study was approved by the institutional ethics committee (approval date: December 22, 2011 and decision number: 2011/88).
Peer-review: Externally peer-reviewed.
Conflict of Interest: He authors declared no conflicts of interest with respect to the authorship and/or publication of this article.


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