Gene 536 (2014) 171–176

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Combined genetic mutations have remarkable effect on deep venous thrombosis and/or pulmonary embolism occurence

Erdal Simsek [a,*,1], Ahmet Yesilyurt [b,*,1], Ferda Pinarli [b], Nilnur Eyerci [b], A. Tulga Ulus [a]

[a] Turkiye Yuksek Ihtisas Training and Research Hospital, Department of Cardiovascular Surgery, Ankara, Turkey
[b] Diskapi Yildirim Beyazit Training and Research Hospital, Department of Stem Cell and Genetic Diagnostic Center, Diskapi, Ankara, Turkey

ARTICLE INFO

Article history:
Accepted 10 November 2013
Available online 12 December 2013

Keywords:
Deep venous thrombosis
Pulmonary embolism
Genetic mutation

ABSTRACT

Purpose: Although deep vein thrombosis and thromboembolic diseases differ among various races, they are still important in our day. The difficulties in treatment and following-up of these diseases are caused by secret genetic mutations rather than predisposing factors.
Methods: Between January 2011 and May 2013, patients who were traced for deep vein thrombosis and/or pulmonary embolism were evaluated retrospectively. 84 patients (53.6% males and 46.4% females) were included in the study. Their family histories, predisposing factors and treatments were researched. Factor V Leiden (G 1691A), Factor II G20210A, Plasminogen Activator Inhibitor-Type 1 (4G/5G), and Methylene Tetrahydrofolate Reductase (C677T, A1298C) mutations were investigated from peripheral venous blood.
Results: Among the genetic mutations we searched, the incidence of single mutation rate was observed at 11.9%, double mutation collocation at 44%, triple mutation collocation at 29.8%, quadruple mutation collocation at 13.1%, and finally, quintuplet mutation collocation at 1.2%. Our approximate mutation number was found as 2.47 ± 0.91.
Conclusion: We observed that multiple mutations were high in number compared to single genetic mutations. The patients who have multiple mutations should be more in the front line considering their diagnosis, treatment and following up, and also in terms of decreasing mortality, morbidity and recurrence.

1. Introduction

Deep Venous Thrombosis (DVT) is such a common thrombosis of deep venous system that it can give out bad clinic results. While DVT is seen in one million people in the United States every year, Pulmonary Embolism (PE), ending in death, could develop between 50 and 20,000 of these cases (Anderson et al., 1991).

Among the most common risk factors that have big roles for DVT are; surgery, cancer, trauma, critical care conditions, advanced age, estroprogestative treatment, immobilization, chronic obstructive pulmonary disease, fractures, pregnancy and post partum conditions. The effects of environmental and genetic factors and venous thrombosis development show differences in intersocietal and individual means.

Venous thromboembolism is seen in every 1/5 of one thousand thrombosis cases yearly (Dahlbäck, 2008). The risk of embolism occurrence differs greatly when the area that has thrombosis, the duration and dose of the treatment, and the reasons that create tendency to thrombosis are taken into account. The genetic defects, which increase tendency to thrombosis, also increase PE risk.

PE may burst into sight by subclinical and symptomatic verities (pleuritic chest pain, wheezing, non-productive cough) (Sode et al., 2013) and it may lead to a very heavy clinical situation which ends in patient's death. While it is easy to doubt for PE in the patients who have had DVT, it is not that easy to establish a final diagnosis in the non-DVT group. That's why, according to the Wells Criteria, which evaluates the patient in terms of anamnesis and clinical conditions (Table 1), it shows less probability of occurrence with the score ≤ 4 (2.3%–9.4% PE risk), score being >4 shows more probability (28%–52% PE risk) (Wells et al., 2000). In the definitive diagnosis, the first choice for imaging method is Computed Tomographic Pulmonary Angiography (CTPA).

Genetic mutations are the important underlying factors which increase the tendency to venous thrombosis and thromboembolism in vast amount. By the reason of mutations, venous thrombosis and

Abbreviations: DVT, deep venous thrombosis; PE, pulmonary embolism; CTPA, computed tomographic pulmonary angiography; FV, Factor V; *FVL*, Factor V Leiden; *PTM*, prothrombin gene; MTHFR, Methylene Tetrahydrofolate Reductase; *PAI-1*, Plasminogen Activator Inhibitor-Type 1; VTE, venous thromboembolism.

* Corresponding authors at: Turkiye Yuksek Ihtisas Training and Research Hospital, Department of Cardiovascular Surgery, Kızılay Street, Altindağ, Ankara, Turkey. Tel.: +90 5323280217.
E-mail addresses: erdaldr@yahoo.com (E. Simsek), dryesilyurt@hotmail.com (A. Yesilyurt), ferdapinarli@yahoo.com (F. Pinarli), helezon2003@gmail.com (N. Eyerci), uluss@yahoo.com (A.T. Ulus).
[1] Authors that contributed equally to this work.

0378-1119/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gene.2013.11.019

Table 1
Wells scoring method.

Criteria	Score
Clinically suspected DVT	3
Alternative diagnosis is less likely than PE	3
Tachycardia (heart rate > 100)	1.5
Immobilization (at least 3 days),or surgery in the previous 4 weeks	1.5
History of DVT or PE	1.5
Hemoptysis	1
Malignancy (with treatment within 6 months) or palliative	1

PE: Pulmonary Embolism, DVT: Deep Venous Thrombosis.

thromboembolic phenomena might occur in the patients who have liability to thrombosis, without having any other predisposing factors.

Factor V (FV) is a coagulation protein which has a cofactor mission in transforming prothrombin into thrombin that causes fibrin generation. As C.1691G>A mutation results, Arginine 506 develops due to replacement with glutamine, leading to the occurrence of Factor V Leiden (FVL) that has tendency to thrombosis. This way, venous thromboembolism, DVT, and pulmonary embolism risks increase just like portal, cerebral and retinal vein thrombosis risks do (Dentali et al., 2008; Rehak et al., 2008).

G20210A mutation in the prothrombin gene (PTM), and the increase in mRNA production due to the transitions among guanine–adenine nucleotides in 20210 position lead to an increase in prothrombin increase thus in thromboembolic disease risk.

Methylene Tetrahydrofolate Reductase (MTHFR) plays a role as an enzyme on the way of transmethylation that makes methionine synthesis in the parts of DNA. Thereby, methylation synthesis decreases in DNA and causes hyper coagulation which progresses together with plasma homocysteine volume. Polymorphism prevails in C677T and A1298C.

Plasminogen Activator Inhibitor-Type 1 (PAI-1) is a specific plasminogen activator inhibitor which is released from endothelium cells, hepatocytes and megakaryocytes. PAI-1 plasma level increases due to its mutation and as this would inhibit fibrinolysis path, an increase in coagulation can be observed.

In our study, FVL, PTM, PAI-1, and MTHFR, which are sub-types of C677T and A1298C,were analyzed. We sought for the effects of mutations in the genes – individually or together – on venous thrombosis and thromboembolism occurence risk.

2. Materials and methods

Throughout a two-centered study done between January 2011 and May 2013, patients who were diagnosed DVT and/or followed up to have PE later on were evaluated retrospectively. The necessary consent was gotten from the ethical committee of the center before the study was begun. The followed patients who had had taken treatment for DVT and/or PE and for genetic mutation research purposes before, constituted up our study group. There were 84 patients and 53.6% of them were males, while 46.4% were females and their approximate age was 46.083 ± 14.51. The patients' DVT, DVT + PE, and PE rates, the classifications according to where thrombosis and embolism developed, predisposing factors, homocysteine levels, DVT and PE relapse rates, genetic mutations with their associations and, finally, family histories were evaluated. Doppler Ultrasonography was used to establish a final diagnosis for DVT, while PE definitive diagnosis was established by CTPA. PE that developed right after DVT was separated into subgroups by defining right, left, and right + left lung involvement.

FVL (G 1691A), PTM, PAI-1 4G/5G, and MTHFR (C677T, A1298C) were checked from peripheral venous blood to search for the efficiency of venous thrombosis and thromboembolism generations. Genetic mutations, their being heterozygote and homozygote, also the rate of their being single or together were evaluated. The effects of these rates on DVT and PE development risk were checked up on.

3. Data collection

Informed consent for genetic analysis was obtained from each individual. It was approved by the local Human Ethics Committee. For each subject, family and patients' clinical data were collected by Diskapi Yildirim Beyazit Training and Research Hospital Genetic Polyclinic.

4. DNA isolation

Genomic DNA of patients and health controls were isolated from 200 μL of peripheral blood samples by using automated EZ1 magnetic bead-based system according to the manufacturer's instructions (Qiagen, Hilden, Germany).

5. Real-time PCR

To show association of thrombophilia mutations of Factor VL gene with MTHFR enzyme gene, PAI-1 and Prothrombin or coagulation Factor II gene were investigated in the patients and controls. The samples were genotyped for FV G1691A, FII G20210A, C677T and A1298C of MTHFR, PAI-1 4G/5G gene mutations by using melting curve analysis (Nuclear Laser Medicine, Settela, Milan, Italy) with Rotor Gene 6000 platform.

6. Statistical analysis

The demographic and clinical parameter frequencies and descriptive analysis were performed. One-way ANOVA test was used to determine the statistical differences according to the number of mutations. p value below 0.05 was considered statistically significant ($p < 0.05$). All values reported were mean ± SD.

7. Results

In the study group that was evaluated retrospectively; 69% DVT, 61.9% PE, and 31% DVT + PE were observed. According to Doppler Ultrasonography results, DVT (Fig. 1), and Tomography results PE were grouped into sub-groups and their distribution ratio was determined. Related to this, it was observed that DVT below knee and right PE was seen more. While DVT was detected at below knee, above knee, below knee + above knee, none of the patients had venous thrombosis at pelvic levels. 6 patients with trauma (7.14%), 6 patients with post partum accident (7.14%), 2 patients with oral contraceptive usage (2.38%), and 7 patients with operation history (8.33%) were detected as a predisposed factor. The family history for venous thrombosis was determined at 9.5% and at 4.8% rates for PE. While the relapse rates for DVT were stated respectively as; once (8.3%), three times (1.2%), four times (1.2%), the relapse rates for PE were as; once (7.1%), twice (7.4%), and three times (1.2%). PE rates were observed at 33.3% ($p > 0.05$) in below knee DVT, 9.5% ($p > 0.05$) in above knee DVT, and at 42.9% rates ($P < 0.05$) in below + above knee DVT.

Homocysteine levels were detected as, 15.6 ± 5.9 μmol/L in patients with heterozygote MTHFR mutation ($p < 0.05$), 22 ± 69 μmol/L in patients with homozygote MTHFR mutation ($p < 0.05$), and 7.5 ± 1.49 μmol/L in patients without MTHFR mutation ($p > 0.05$).

The percentages of sub-groups according to the number of mutations are shown in Table 2. Percentages of single and combined mutations are shown in Fig. 2. In accordance with this, it is determined that double mutations are the highest in number. Mutations and their subgroup divisions are shown in Fig. 3. In relation to this, PAI-1 4G/5G, MTHFR C677T, and MTHFR A1298C heterozygote mutations are encountered more.