The Influence of Thrombophilic Gene Mutation on Recurrence of Venous Thromboembolism: A Retrospective Cross-Sectional Study

Tekrarlayan Venöz Tromboemboli'ye Trombofilik Gen Mutasyonunun Etkisi: Geriye-Dönük Kesitsel Bir Araştırma

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Abstract: The aim of this study is to evaluate the influence of the risk factors from the point of thrombophilic gene mutations (TGM) with type and inter-cooperation's on recurrence in venous thromboembolism (VTE) patients.This retrospectively and cross-sectionally designed study was conducted between 2008–2009. The VTE patients, who were evaluated for TGM were elected. Among a total of 109 patients, the mean age at first VTE event was 42.6±14.1 years. Fifty-nine (54.1%) patients were male. While 33 (30.3%) patients had primary VTE, 46 (42.2%) patients had recurrent VTE (rVTE). In the univariate analysis, the significant variables associated with the increased rate of rVTE were age ≥40 years, first event at in-hospital, malignancy, internal medical disease, TGM, factor V Leiden, prothrombin G20210A. The analyses of different mutation count on rVTE pointed that, there were significant differences in recurrence rates, except groups with no mutation and one mutation. Additionally, the increasing number of clinical risk factors and TGM per case were considerably associated with rVTE in both univariate and multivariate analysis. Regarding the risk of rVTE, the TGMs were significant but do not appear to play a vital role per se. However, simultaneous existence of clinical risk factors, including TGM seem to be more important for the prediction of rVTE.
Key Words: venous thromboembolism, recurrence factor V leiden, prothrombin G20210A, methylenetetrahydrofolate reductase C677T and A1298C.

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Özet: Bu çalışmanın amacı, trombofilik gene mutasyonu (TGM) tipi ve birlikteliği açısından tekrarlayan venöz tromboemboli (rVTE) hastalarında risk faktörlerinin etkinliğinin değerlendirilmesidir. Geriye-yönelik ve kesitsel olarak tasarlanan bu çalışma, 2008-2009 yılları arasında gerçekleştirildi. TGM açısından tetkik edilen VTE hastaları çalışmaya alındı. Toplam 109 hastada ilk VTE atak yaşı ortalaması 42.6±14.1 yıl idi. Ellidokuz (%54.1) hasta erkekti. Otuzüç (%30.3) hastada birincil VTE mevcut iken 46 (%42.6) hastada rVTE bulunmaktaydı. Tek değişkenli analizde artmış rVTE oranı ile belirgin birliktelik gösteren değişkenler yaş ≥40 yıl, ilk atağın hastane-içi'nde olması, malignite, dahili hastalık, TGM, faktör V Leiden ve protrombin G20210A mutasyonu idi. Farklı mutasyon sayılarının rVTE için analizi, mutasyonu olmayan ve tek gen mutasyonu olan hastalar haricinde belirgin farklılık göstermektedir. Ek olarak, artan hasta başına klinik risk faktörü ve TGM sayısı rVTE ile hem tek değişkenli hem de çok değişkenli analizlerde belirgin ilişkili saptandı. Tekrarlayan VTE açısından TGM'ları, rVTE riskini arttırmakta, ancak tek başlarına önemli bir rol oynamıyor gibi görünmektedir. Ancak, TGM da dahil olmak üzere klinik risk faktörlerinin eş zamanlı birlikteliği, rVTE'nin öngörüsünde daha önemli görünmektedir.
Anahtar Kelimeler: venöz tromboemboli, tekrarlayan faktör V leiden, protrombin G20210A, metilentetrahidrofolat redüktaz C677T, metilentetrahidrofolat redüktaz A1298C.

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1. Introduction

Venous thromboembolism (VTE) is the leading cause of considerable morbidity and mortality worldwide (1,2). Moreover, it results in loss of employment-power and consumption with large economic burden. Other than a previous VTE event, the existence of a thrombophilic gene mutation (TGM) as a subject of influencing factor on recurrent VTE (rVTE) was proved(1-6), On the other hand, the TGM analysis performed at the first VTE episode does not indicate rVTE and gain favour for the patient(4-6).The co-existence of clinical risk factors are reported to be more determinant than TGM alone(4). In this study, in the foreground of the influence of TGM, we evaluate the risk factors associated with rVTE.

2. Materials and Methods

This analytic study was designed retrospectively and cross-sectionally. Ethical approval was obtained from the Local Evaluation Committee of Non-invasive Clinical Research at Dokuz Eylül University Faculty of Medicine on 28 June 2010 (Nr: 2010/05-06). Between 2008 and 2009, the hospital archive was searched until 2004. As pointed in the inclusion criterion cases, who have been evaluated for TGM were elected. Informed consent was taken orally through phone call from the case him/herself or from their relatives if they were not alive. Then the remaining designated data were collected.

Data Collection and Inclusion Criteria

The cases of whom; (1) the application and the diagnosis of the first episode was made in our department, (2) the diagnosis of deep vein thrombosis (DVT) was made with lower/upper extremity duplex ultrasonography, (3) the diagnosis of pulmonary embolism was made with CT angiography and/or pulmonary ventilation – perfusion scintigraphy, (4) the diagnosis of renal, hepatic, serebral and mesentery vein thrombosis was made with duplex sonography and/or CT angiography, (5) the out-patient follow-up data in the archive is regular, took at least 3 years and includes physical examination with d-dimer levels, (6) the diagnosis of rVTE was made with one of the above-mentioned objective diagnostic means.

Study Variables

The demographic and clinical characteristics of the patients were collected. Throughout the article, 'age' implies 'the age at the first VTE episode'. According to Van Cott et al.(7), the patients were classified as primary VTE (pVTE). Diagnosis of rVTE was made clinically, which was also confirmed by imaging modalities. As a clinical protocol, patients with the first VTE event were anticoagulated with warfarin sodium for at least 6 months. The patients with rVTE or whose first event was a complicated pulmonary embolism received lifelong anticoagulation.

The TGM analyses were performed using Amplitronyx 6 Thermal Cycler with 384-Well Block (Nyx Technik, Inc., San Diego, CA, USA). While the factor V Leiden (FVL) analysis was performed using allele-specific polymerase chain reaction (PCR), the prothrombin G20210A (PT) was evaluated by PCR with restriction enzyme digestion, and methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C were analysed by PCR with restriction enzyme analysis.

Statistical Analysis

The statistical analyses were performed using a licensed Statistical Package for Social Sciences (Kaysville, Utah, USA), version 15.0. The distributions were presented as frequency and percent. Because the continuous variables were normally distributed, the data were presented as mean ± standard deviation. The continuous variables were compared using independent samples t test. The comparison of categorical variables was done using Chi-square test or Fischer's exact test. The multivariate analysis was performed through forward logistic regression analysis. A p value of ≤ 0.05 was considered significant.

3. Results

The study comprised a total of 109 patients. The demographic and clinical characteristics of the patients are presented in Table 1. Most of the patients (85%) had their VTE as DVT at out-hospital (80%). While 33 (30.3%) patients were classified as pVTE, 46 (42.2%) patients had rVTE.

The patients, whose first event was in-hospital (47.6±12.3 years) were older than that of others whose first event was out-hospital (40.8±14.2 years) [t(107)=2.29, p=0.024]. The patients with pVTE (31.8±10.6 years) were younger than the patients with secondary VTE (47.3±12.8 years) [t=6.1, 107 df, p<0.001]. Primary VTE cases were also highly found (90.9%) to have their first event out of hospital [χ^2=7.43, 1 df, p=0.006]. Other than 10 (9.2%) patients who did not found to have TGM, the rate of TGM were 90.8%.

The distribution of the ascertained risk factors is presented in Table 2. As the history of VTE was the most common, internal medical disease was found to be the second most common determined risk factor of VTE. When the influence of the total number of risk factors per case to the location of the first event was taken into consideration, it was found that with the increasing number of total risk factor, the rate of suffering the first event in-hospital has significantly increased [χ^2=25.5, 5 df, p<0.001].

The total number of TGM per case and the distribution of it is presented in Table 3. While heterozygous MTHFR C677T was the most common, homozygous PT was the least common TGM. It was found that the cases with rVTE were 45±12 years old and it did not significantly differ from others without recurrence (41±15 years) [t=-1.75, 107 df, p=0.08] (Table 4). On the other hand, the rVTE rate was significantly higher in patients who are ≥40 years of age [χ^2=5.57, 1 df, p=0.018]. Gender did not constitute a significant difference regarding recurrence [χ^2=2.3, 1 df, p=0.13]. It was found that the patients, whose first episode had occurred in-hospital revealed significantly higher rVTE rate [χ^2=8.8, 1 df, p=0.003]. The pVTE did not constitute significant difference in recurrence rate [χ^2=0.15, 1 df, p=0.69]. The increase in total number of risk factors was found to have a significant effect on recurrence rates [p<0.001]. The rate of rVTE in patients with TGM [χ^2=4.68, 1 df, p=0.042] was higher. While the rate of FVL [χ^2=13.2, 1 df, p<0.001] and PT [χ^2=3.17, 1 df, p=0.07] were significantly higher in cases with rVTE, the rate of MTHFR C677T [χ^2=3.26, 1 df, p=0.07] and MTHFR A1298C [χ^2=1.53, 1 df, p=0.21] were slightly higher but not significant. Additionally, the increase in TGM number per case was also found to have a considerable influence on recurrence rates [p<0.001].

The influence of TGM count per patient, type and collocation on recurrence are revealed in Table 5. The analyses of different mutation count on recurrence pointed that, there were significant differences in recurrence rates, except groups with no mutation and one mutation (p=0.25). In subgroup analysis of comparison between patients without TGM and patients with different TGMs, it seems that none of the mutation type alone had a significant effect on recurrence. FVL and PT seems to have an influence on recurrence when accompanied by MTHRF.

With the increasing total risk factor per patient, the risk of in-hospital VTE occurrence increased [(3, N=109) = 64.27; p<0,001]. The mean total number of risk factor per case in patients who experienced their first VTE episode in-hospital and out-hospital were 4.0±1.1 and 2.7±1.3, respectively [*t*(107) = 4,9; p<0,001].

Logistic regression analysis of the influencing factors on rVTE was commenced but, because history of surgery, medical illness and malignancy were aroused with total risk factors, they were not included in the same model. The results of logistic regression analysis in two models were presented in Table 6.

Table 1.
Demographic and clinical characteristics of the patients.

Characteristic	n	%
Age at First Event (mean ± SD)	42.6 ± 14.1 years	
Age Groups		
<40 years	45	41.3
≥40 years	64	58.7
Male Gender	59	54.1
Place of First Event		
In-hospital	29	26.6
Out-hospital	80	73.4
VTE type		
DVT	85	78
PE	9	8.3
DVT + PE	15	13.8
Primary VTE	33	30.3
Recurrent VTE	46	42.2
Thrombophilic Mutation		
MTHFR included	99	90.8
MTHFR excluded	48	44

Abbreviations: DVT: deep vein thrombosis, MTHFR: methylenetetrahydrofolate reductase, PE: pulmonary embolism, SD: standart deviation, VTE: venous thromboembolism.

Table 2.
The distribution of the risk factors for VTE.

Risk factors	n	%
History of VTE	46	42.2
Internal medical disease[I]	27	24.8
Varicose veins	27	24.8
History of surgery[II]	26	23.9
History of trauma[IV]	17	15.6
Use of OC	15	13.8
Family history for VTE	14	12.8
Malignancy[I]	11	10.1
Lower extremity paresia/plegia	9	8.3
Pregnancy	8	7.3
Central vein catheterization	7	6.4
Travel >6 hrs	7	6.4
Transvenous pacemaker	2	1.8
Total risk factors		
≤3	67	61.5
≥4	42	38.5

Abbreviations: MTHFR: methyleneterahydrofolate reductase, OC: oral contraceptive, VTE: venous thromboembolism.

[I] *Internal medical disease including malignancy, but not including thrombophilic mutation.*

[II] *History of surgery including patients with their initial VTE event occurred within 3 months of their surgery whether it was in-hospital or out-hospital.*

[IV] *History of trauma including patients who experienced minor trauma without necessitating surgical intervention, but plaster applied resulting in immobile extremity within the last 3 months.*

Table 3.
Thrombophilic mutation characteristics of the patients.

Thrombophilic Mutation	n	%
FVL	36	33
Hom.	9	8.3
Het.	27	24.8
PT G20210A	16	14.7
Hom.	3	2.8
Het.	13	11.9
MTHFR	89	81.7
C677T	65	59.6
Hom.	16	14.7
Het.	49	45
A1298C	47	43.1
Hom.	7	6.4
Het.	40	36.7
Number of TM Per Patient		
0	10	9.2
1	48	44
2	38	34.9
≥3	13	11.9

Abbreviations: FVL: factor V Leiden, Het: heterozygous, Hom: homozygous, MTHFR: methylenetetrahydrofolate reductase, PT: prothrombin, TM: thrombophilic mutation, VTE: venous thromboembolism.

Table 4.
The univariate analysis of variables associated with recurrent VTE.

	Recurrent VTE		
Variable	**Yes (N=46) (n, %)**	**No (N=63) (n, %)**	**p value**
Age groups			
<40 years	14 (29.8)	31 (50)	**0.018**$^{\alpha}$
≥40 years	33 (70.2)	31 (50)	
Gender			0.130$^{\alpha}$
Male	21 (45.7)	38 (60.3)	
Female	25 (54.3)	25 (39.7)	
First event place			**0.003**$^{\alpha}$
In-hospital	19 (41.3)	10 (15.9)	
Out-hospital	27 (58.7)	53 (84.1)	
Primary VTE	13 (28.2)	20 (31.7)	0.690$^{\alpha}$
Secondary VTE	33 (71.7)	43 (68.3)	
Malignancy	8 (17.4)	3 (4.8)	**0.031**$^{\beta}$
History of surgery	18 (39.1)	8 (12.7)	**0.006**$^{\alpha}$
Internal medical disease	19 (41.3)	8 (12.7)	**0.003**$^{\alpha}$
Total risk factor per case			**<0.001**$^{\beta}$
1	0 (0)	10 (15.9)	
2	1 (2.2)	21 (33.3)	
3	11 (23.9)	24 (38.1)	
≥4	34 (73.9)	8 (12.7)	
Genetic mutation	43 (93.5)	56 (88.9)	**0.042**$^{\beta}$
FVL	27 (58.7)	9 (14.3)	**<0.001**$^{\alpha}$

PT G20210A	11 (23.9)	5 (7.9)	**0.007**[α]
Total GM per case			
0	1 (2.2)	9 (14.3)	
1	13 (28.3)	35 (55.5)	**<0.001**
2	21 (45.6)	17 (27.0)	
≥3	11 (23.9)	2 (3.2)	

Abbreviations: VTE: venous thromboembolism.
[α]Chi-square test, [β]Fischer's exact test.

Table 5.
The influence of thrombophilic mutation count per case, type and collocation on recurrent VTE.

Mutation Characteristics	χ^2	n	p value
Mutation count per case			
0 – 1	1.32	58	0.25
0 – 2	6.53	48	**0.01**
0 – ≥3	12.61	32	**<0.001**
1 – 2	7.05	86	**0.008**
1 – ≥3	14.19	61	**<0.001**
Mutation type/collocation			
0 – FVL	3.20	16	0.1
0 – PT	0.96	13	0.4
0 – C677T	1.31	35	0.4
0 – A1298C	0.10	24	1
0 – FVL+MTHFR	5.60	28	**0.04**
0 – FVL+C677T	5.50	22	**0.03**
0 – FVL+A1298C	3.20	16	0.12
0 – PT+MTHFR	6.80	17	**0.03**
0 – PT+C677T	2.70	14	0.17
0 – PT+A1298C	8.77	13	**0.01**
0 – C677T+A1298C	2.75	22	0.16
0 – PT+C677T+A1298C	12.8	20	**<0.001**

Table 6.
Multivariate analysis result of risk factors associated with recurrent VTE.

Variable	β (SE)	Odds ratio	p value	95% CI
Model 1				
Male gender[α]	0.98 (0.76)	2.67	0.20	0.6 – 11.9
Age ≥40 years[α]	2.21 (0.85)	9.1	0.01	1.7- 48.4
Total risk factor per case (0-5)[β*]	2.9 (0.62)	18.4	<0.001	5.4 – 62.6
Total mutation per case (0-4)[β]	1.9 (0.59)	6.7	0.001	2 – 21.7
Model 2				
Age ≥40 years[α]	0.49 (5.54)	1.64	0.36	0.56 – 4.76
History of surgery[α]	2.05 (0.66)	7.7	0.002	2.1 – 28.3
Internal medical disease[α‡]	2.12 (0.65)	8.3	0.001	2.3 – 29.8
Malignancy[α]	2.16 (0.88)	8.7	0.015	1.5 – 49.5
Total mutation per case (0-4)[β]	1.7 (0.4)	5.5	<0.001	2.5 – 12.1

Abbreviations: CI: confidence interval, SE: standard error. Multivariate analysis included
[*]TM was not included. [α] indicates categorized, [β] indicates continuous variables. [‡] Does not include patients with malignity.

4. Discussion

The literature comprises a substantial number of studies that have investigated the risk factors, including the TGMs that may play a key role for rVTE(1, 4, 5, 8-13). Although presence of TGM as a risk factor for the index VTE event is undisputed, its impact on the rVTE is a highly debated issue(1, 4, 5, 9-12, 14-19). Although either FVL, PT or even the double heterozygous carriers of both alterations were not found to be a risk factor for a rVTE event in some studies(4, 5, 20), it was found in two prospective trials that in comparison to noncarriers, patients with heterozygous FVL had an increased risk of recurrence(21, 22). The PT was also reported to be a risk factor for rVTE(3, 14). Additionally, MTHFR mutations are usually indicated as a compounding risk factor when associated with FVL or PT mutation (3). Contrary to our results, Baglin et al.(4) did not found a considerable influence of either FVL or PT on recurrence. However, FVL and PT alone was found to have a considerable influence on recurrence in the current study. Additionally, the MTHFR C677T and MTHFR A1298C did not constitute a significant risk for recurrence unless combined with FVL or PT, which also supported the findings of Almawi et al(3).

As reported by Almawi et al.(3), the presence of more than one TGM was considerably associated with the risk of VTE. In our patient cohort, in comparison to the patients without TGM, the rate of rVTE was not significantly higher in patients with one TGM. In subgroup analysis of the patients with none or one TGM, it was found that the recurrence rate did not differ considerably in any of the TGMs. It became significant when the TGM count per patient was more than one which also supported the findings of Almawi et al(3). In comparison to the patients with one TGM, the rVTE rate was even higher in patients with two or more TGMs.

Patients with pVTE are suggested to be tested for TGM(7). On the other hand, regarding recurrence, the literature includes conflicting evidence for significance of the clinical risk factors other than the TGMs per se(4-6). In the current series, 33 (30.3%) of patients had pVTE, and supporting the literature, the total number of risk factor per case came into prominence in our patient cohort(4-6). Baglin et al.(4) emphasized that the clinical risk factors are more determinant than TGM because, the TGM analysis which was utilized at the first VTE episode does not indicate rVTE within the first two years. Having almost the same opinion, Christiansen et al.(5) and Mazzolai et al.(6) have emphasized that the TGM analysis performed at the first VTE episode does not favourably gain favour for the patient. As revealed in Table 4, with the increasing risk factor count per patient, the rate of recurrence considerably increased. Our results also supported the higher significance of the accompanying clinical risk factors other than the TGM. This significance can be explained through the finding that the rate of patients who were ≥40 years old at first VTE event and whose first event occurred in-hospital were higher in patients with rVTE (Table 4). The patients whose first event was in-hospital were also found to be older than the patients whose first event was out-hospital. When the distribution of the clinical risk factors (Table 2) were considered, depending on the age, older patients seem to have more clinical risk factors for rVTE, which led the co-existence of clinical risk factors more prominent as a predictor of rVTE.

Because it was emphasized that the analysis for TGM does not gain benefit at the first event as an indicator for rVTE, it is not recommended in patients whose first VTE event was a pVTE(5-7). As the departmental strategy, all patients with VTE had been evaluated for TGM at first VTE event. In our patient cohort, the TGM rates, either MTHFR mutation was included or not, were 90.8% and 44%, respectively. Although this high rate of TGM gave rise to thought that TGM should be performed to all patients at the time of first VTE event, the rate of pVTE in patients with recurrence was not significant in our results, which supported the literature regarding the

inefficacy of TGM on recurrence(4-6). The pVTE patients were younger and experienced their first VTE episode out-hospital. Additionally, the total number of risk factor per patient in patients with primary VTE was also lower. In our opinion, this finding additionally supported the significance of the combination of risk factors as a contributor for rVTE.

Although determination of protein-C, protein-S and antithrombin-II deficiency is part of thrombophilia testing(7), it is a common notion in clinical practise that their level decrease during the acute phase of VTE which necessitates delaying of their testing 3 months after the acute episode and after withdrawal of warfarin treatment(6, 7). According to the above-mentioned reasons, these laboratory parameters were not included in the study which, in our opinion a scare side of the study.

In this study, the reported rate of rVTE was objectively diagnosed. Additionally, the VTE in all the patients comprising the study population was symptomatic, which indicated that the diagnosis of rVTE in asymptomatic patients would have been missing. Missing the rVTE events due to an asymptomatic clinical state was emphasized in the literature as a confounding situation(1), In our opinion, this situation had an inevitable impact on the rates of rVTE, which was thought to be higher than it was revealed herein. Other than the retrospective design of the study, the likelihood of missing the patients with asymptomatic rVTE were the major limitations of the current study.

In summary, the existence of TGM has a confirmed impact on the recurrence of VTE. Regarding the risk of rVTE, the TGMs per se do not appear to play a key role. However, simultaneous existence of clinical risk factors, including TGM, which would be interpreted as a contributing factor of a recurrent event, was proved to be more important for the prediction of rVTE.

Note: Some of the data of this study was presented as oral presentation at the 7th Congress of Update in Cardiology and Cardiovascular Surgery in association with TCT Mediterranean; 24-27 March 2011, Antalya, Tukey. The Heart Surgery Forum 2011;14(Suppl-1):41-42.

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