Original Article

Evaluation of thrombophilic risk factors in patients diagnosed with pulmonary embolism: Single center experience

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ABSTRACT

Objective: To evaluate the thrombophilic risk factors and incidence in patients presenting at the hematology outpatient clinic for further investigation after the diagnosis of pulmonary embolism.

Methodology: A total of 15 cases (8 male, 7 female) with the diagnosis of pulmonary embolism and referred to hematology clinic for investigating thrombophilic risk factors were retrospectively evaluated. Thrombophilic screening tests for these patients are as follows: factor V leiden (FVL), prothrombin G20210 A (PTG) and methylentetrahydrofolate reductase (MTHFR C677 T) gene mutations, protein C (PC), protein S (PS) and antithrombin III (AT III) deficiency, active protein C resistance (APC-R), antinuclear antibodies (ANA), anti dsDNA, anti-cardiolipin antibodies IgM and IgG, lupus anticoagulant, homocysteine and factor VIII levels were investigated.

Results: The commonest thrombophilic defect was MTHFR C677T gene mutation (n=12) (80%). Single defect (n=4) was found at a rate of 26.6%, double defects (n=2) 13.3%, triple defects (n=5) 33.3% and four defects (n=3) 20%. One patient had no defects.

Conclusion: In this retrospective study carried out in patients with pulmonary embolism, MTHFR gene mutation was found to be the commonest cause for hereditary thrombophilia as a single risk factor and/or together with other thrombophilic risk factors.

KEY WORDS: Pulmonary embolism, Thrombophilia, Factor V leiden mutation, Methylentetrahydrofolate reductase gene mutation.

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INTRODUCTION

Increased risk for thrombosis is known as hypercoagulability/thrombophilia. hrombophilia is defined as the common name of a group of hereditary and acquired conditions.[1,2] Thrombophilia can also be defined as a tendency for clot formation in unusual sites. Thrombophilia is an important cause of mortality and morbidity.[3,4]

In hereditary thrombophilia, there is a genetic tendency for venous and/or arterial thromboembolism. The commonest cause of hereditary thrombophilia is FVL mutation and is responsible for 40%-50% of cases. PT G20210 A gene mutation, PC, PS and AT III deficiency can be cited among other causes of hereditary thrombophilia.[5] Thrombosis

can occur at an early age and more frequently in the co-existence of a homozygous mutation with two or more heterozygous mutations.[6]

Venous thromboembolism (VTE) can appear with the clinical picture of pulmonary embolism (PE) and deep vein thrombosis (DVT). VTE is one of the commonest causes of cardiovascular mortality and morbidity.[7,8] Pulmonary embolism most frequently arises from the thrombosis occurring in the deep veins of the lower extremities. Delay in the diagnosis and treatment of PE leads to a marked increase in mortality and morbidity.[9]

The purpose of this study was to evaluate the thrombophilic risk factors and incidence in patients with the diagnosis of pulmonary embolism referred to the hematology clinic.

METHODOLOGY

In the study, a total of 15 cases referred to the hematology outpatient clinic of Diskapi Yildirim Beyazit Training and Research Hospital during a one-year period between January 2010 and January 2011 with the diagnosis of pulmonary embolism were retrospectively evaluated.

At presentation, the patients were assessed whether they had an underlying co-morbid condition causing a tendency for thrombosis. Patients with diabetes, dyslipidemia, recent history of surgical intervention, central venous catheter, malignancy, myeloproliferative disease, paroxysmal nocturnal hemoglobinuria (PNH), autoimmune diseases like Behcet's Disease or those on oral contraceptives and hormone replacement therapy increasing the risk of thrombosis were excluded from evaluation. Patients were asked about a previous history of DVT & thromboembolism. Family history of thrombosis was inquired. PC and PS levels were investigated in patients taking coumadine four weeks after cessation of treatment. AT III levels were investigated in patients using heparin or its derivatives 15 days after cessation of treatment. Patients with a history of acute thrombosis underwent investigation for thrombophilia three months later (after the acute phase passed). Positive results were repeated.

Regarding etiology of thrombophilia, patients were tested for PC activity measurement, total and free PS antigen and activity, AT III activity, APC resistance, FVL G1691A, MTHFR C677T and PT G20210A gene mutations, factor VIII level, homocysteine; antiphospholipid antibodies (anticardiolipin antibodies IgG and IgM), ANA, Anti dsDNA, lupus anticoagulants (screening and confirming) were tested for acquired thrombophilia.

FVL and PT G20210A were studied with Roche kits and MTHFR C677T gene mutation was investigated with artus MTHFR LC PCR (QIAGEN) kit. Tests were evaluated with the 'LightCycler 1.5 ROCHE (Real Time PCR unit)' unit. For the investigations of PC, PS, AT III, lupus anticoagulants, APC resistance and factor VIII levels, blood was drawn into citrated (105 molar) tubes after 12-hour fasting. APC-R (Dade Behring proC global kit), PC (Dade Behring), PS (Dade Behring), ATIII (Dade behring), FVIII (Dade Behring) kits were used in the study. . Blood samples were taken into 105-molar citrated tubes and were centrifuged for 15 minutes at 1500xg. They were kept at -20C until the study. Samples were analyzed with the Dade Behring BCS unit. Anticardiolipin antibodies (IgG and IgM) were searched at the microbiology (ELISA) laboratory of our hospital using the Biomaster unit and with Intec cardiolipin IgG and IgM kits.

Statistical Analysis: Statistical analysis of the data was carried out using the SPSS 11.5 packet program. Data were given as mean ± Standard deviation. Chi-square test was used for comparisons. Level of statistical significance was taken as $p<0.05$.

RESULTS

A total of 15 cases with the diagnosis of PE were included in the study. General characteristics of the patients are shown in Table-I. The most commonly detected thrombophilic defect was MTHFR C677 T gene mutation (n=12, 8 heterozygous, 4 homozygous) with a rate of 80% and other detected defects were FVL mutation (n=7, 5 heterozygous, 2 homozygous) with a rate of 46.6% and APC-R (n=5, 33.3%).

Single defects were found at a rate of 26.6% (n=4), double defects 13.3% (n=2), triple defects 33.3% (n=5) and four defects at 20% (n=3). One patient had no defects. Distribution of single and combined defects are shown in Table-II. Four cases (26.6%) had a personal history and one case (6.6%) had a family history of thrombosis.

DISCUSSION

VTE is a frequently seen vascular pathology with numerous environmental and genetic factors playing a role in its pathogenesis. DVT and PE are two important clinical findings commonly seen in VTE. Annual incidence of VTE varies between 0.1% and 0.5%.[10,11] It is known since 1965 that thrombophilic defects are risk factors for venous thrombosis. These hereditary thrombophilic defects are AT III, PC and PS deficiency, FVL mutation, PT G20210A gene

Table-I: General patient characteristics.

Age (years)	*45.06 (21–59)*
Gender	n=15
Female	7 (46.6 %)
Male	8 (53.3 %)
Personal history of thrombosis	4 (26.6 %)
Family history of thrombosis	1 (6.6 %)

mutation, factor VIII, IX and XI elevation, TAFI (thrombin activatable fibrinolysis inhibitor) and hyperhomocysteinemia as a metabolic thrombophilic defect.[12,13] While the incidence of these defects in the normal population is around 25%, its incidence in patients with venous thrombosis is greater than 60%.[14]

PE most commonly arises from the thrombosis in the deep veins of the lower extremities. Delay in the diagnosis and treatment of PE leads to a marked increase in morbidity and mortality.[9] PE is responsible for approximately 10% of hospital deaths.[15] The most commonly detected mutations in thromboembolic diseases are: FVL mutation, PT G20210A gene mutation, PC, PS and AT III deficiencies. FVL mutation is present in 5% of the white race. While in heterozygous FVL mutation there is a seven-fold increase in VTE risk, in homozygous mutation this risk is increased 80-fold.[16] Also in this study, heterozygous FVL mutation in cases with the diagnosis of pulmonary embolism was in the second order with a rate of 46.6%.

FVL mutation is a genetic disorder leading to an increase in the risk of VTE. At the same time, it is a disease characterized with a poor anticoagulant response to APC. The most commonly seen thrombophilic pictures in FVL mutation are DVT and PE. Thrombosis is more often seen in unusual areas. FVL mutation also causes a 2- or 3-fold increase in miscarriage risk and other possible obstetric complications.[17] It was shown in a study that FVL mutation and PT G20210A gene mutation are high risk factors for PE and DVT and may lead to repeating thromboembolic events. FVL mutation is particularly seen more frequently in younger patients and consequently thromboembolic events may be seen at earlier ages.[18,19] When a homozygous mutation is combined with one or more heterozygous mutations, thrombosis may occur more frequently and at earlier ages.[6]

In a study by Tug et al. carried out on 188 patients, there were one or more mutations in 179 (95.2%) patients. In this study, the rate of FVL G1691A mutation was 11.7%, that of PT G20210A gene mutation 2.5% and of MTHFR (C677T) gene mutation 30.4%. Again in this study, the most frequent gene mutation detected in 27 cases of pulmonary embolism was MTHFR C677T gene mutation with a rate of 37%.[20] Also in our study, cause of thrombophilia was detected in 14 of 15 cases with the diagnosis of PE (93.3%). Again in PE cases, the most frequent mutation was the MTHFR C677T gene mutation seen at a rate of 80%. This result is compatible with the study of Tug et al.

In a study carried out in 92 cases with the diagnosis acute pulmonary embolism, FVL mutation was detected at a rate of 23.9% (22/92), PT G20210A gene mutation at 3.3% (3/92) and MTHFR gene

Table-II: Distribution of single and combined defects observed in cases diagnosed with pulmonary embolism.

	Male		*Female*		*Total*	
	Number	*%*	*Number*	*%*	*Number*	*%*
Protein C deficiency	1	6.66	2	13.33	3	20
Protein S deficiency	1	6.66	1	6.66	2	13.33
MTHFR	6	40	6	40	12	80
PT.20210 A Gene Mut.	1	6.66	1	6.66	2	13.33
FV Leiden Mut.	3	20	4	26.66	7	46.66
Active PC Resistance	2	13.33	3	20	5	33.33
Factor VIII Elevation			1	6.66	1	6.66
APS			2	13.33	2	13.33
PC + MTHFR	1	6.66	1	6.66	2	13.33
PS + MTHFR	1	6.66			1	6.66
PS + FV Leiden mut.			1	6.66	1	6.66
MTHFR + Prot 20210	1	6.66	1	6.66	2	13.33
MTHFR + FV Leid.	2	13.33	3	20	5	33.33
PT 20210 + FV Leid.			1	6.66	1	6.66
PS + APC resis.			1	6.66	1	6.66
MTHFR + FVIII elev.			1	6.66	1	6.66
MTHFR + APS			2	13.33	2	13.33
MTHFR + Prot20210 + FVLeid.			1	6.66	1	6.66

mutation at 52.2% (48/92). On the other hand, in 99 cases in the control group, FVL mutation was detected at a rate of 12.1% (12/99), PT G20210A gene mutation at 0% (0/99) and MTHFR gene mutation at 53.5% (53/99). While there was a statistically significant difference in this study between the patient and control groups regarding FVL mutation, there were no significant differences in MTHFR and PT G20210A gene mutations.[21] While there was a positive correlation between FVL and PT G20210A gene mutations and VTE in a study of 149 cases, there was no significant correlation between MTHFR gene mutation and VTE.[22]

In a study by Kupeli et al. carried out in the Turkish population, 80 patients with VTE and 104 controls were included in the study. Heterozygous FVL mutation was found at a rate of 6.3%, heterozygous PT G20210A mutation at 6.9% and MTHFR C677T mutation at 23.8% in patients with VTE as a result of this study. It was concluded in this study that thrombophilic risk factors and their incidences may be different in every population.[23]

The relationship between gender and VTE has not been shown.[22] While some studies report that there is no difference between males and females in the incidence of PE,[24] another study has shown that the incidence of VTE is higher in men,[25] and in the ICOPER study, PE was seen more frequently in women (55%).[22,26] As for our study, although 53.3% of the patients were male, there was no significant relationship between PE and gender.

Hyperhomocysteinemia is a weak risk factor for VTE. Studies have shown no difference between patient and control groups. Moreover, checking homocysteine levels is not recommended in the routine screening for thrombophilia because of being influenced by factors such as age, gender, nutrition and vitamin levels.[22]

In all thrombophilic patients with combined genetic disorders, the thrombosis tends to recur. Risk of recurrence is especially higher in PC, PS and AT III deficiencies and in homozygous FVL mutations.[4,22] In our study, combination of MTHFR with FVL mutation was the most frequently encountered (33.3%). Single defect was detected at 26.6%, double defects at 13.3%, triple defects at 33.3% and four defects at 20%.

In conclusion, MTHFR gene mutation may be the most frequently encountered hereditary thrombophilic factor in cases of pulmonary embolism in the Turkish population. In cases referred to hematology outpatient clinics for investigating the etiology of PE, it may be recommended to search primarily for MTHFR and FVL gene mutations which are two most frequently observed thrombophilic defects. Each population should determine its own thrombophilic risk pool due to the heterogeneity. Thus, determination of thrombophilic risk factors decreases ordering for unnecessary analyses, enabling the development of a cost-effective approach.

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