

The Effect of Genetically Related Risk Factors on the Recurrence Rate of Acute Pulmonary Embolism in a Tertiary Teaching Hospital in Jordan.

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Abstract

Objectives: To identify the recurrence rate of Pulmonary Embolism (PE) during the first 6 months of the diagnosis of the patients while they were on anticoagulation therapy in order to study the impact of hereditary risk factors on the recurrence rate in patients with acute pulmonary embolism during the same period.

Methods: A prospective study was conducted at Jordan University Hospital, from January 2005 to the end of December 2007. A follow up was conducted till July 2008. Ninety (90) patients with acute PE were investigated; only 72 patients were included in the study due to the loss of follow up of other patients. All patients were investigated for the genetically related thrombophilic factors (FVL, FII and MTHFR), plasma level of free protein C, protein S, and antithrombin III. The patients were divided into two groups: first group those who have recurrence of PE and the second group those who have no recurrence.

Results: Seven patients (9.7%) out of 72 who met the inclusion criteria, had a recurrent episode of PE within the first 6 months of diagnosis. There was a significant correlation between the recurrence rate of PE and protein C deficiency p value 0.025. There was no significant correlation between the recurrence of PE and the rest of the hereditary thrombophilic factors.

Conclusion: The results of our study necessitate the need to test patients with unprovoked vein thrombosis for the presence of deficiencies of natural anticoagulants; especially protein C level. Other potential risk factors for the recurrence of Venous Thromboembolism (VTE) have to be individualized. Further studies with a larger number of patients are needed to clarify the significance of these risk factors in the recurrence of PE.

Keywords: Genetically Related Risk Factors, Recurrence Rate, Acute Pulmonary Embolism.

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Introduction

Pulmonary Embolism (PE) is a multifactorial vascular disease and the risk of venous

thromboembolism is related to the presence or absence of specific risk factors, these risk factors are environmental and genetic, interacting dynamically.^{1,2} PE is fatal in 4-9% of cases^{3,4}

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and causes 10% of death in hospitalized patients.⁵

The most important genetic risk factors for pulmonary embolism are deficiencies in protein C, protein S, antithrombin III and factor V Leiden or mutation of prothrombin factor and Methyl Tetra Hydrofolate Reductase (MTHFR).^{6, 8} These and other acquired hypercoagulable conditions were found to place patients at higher risk for developing a first episode of Venous Thromboembolism (VTE).^{7, 8} Their role in recurrent VTE remains uncertain, for example the degree of association between FVL and the recurrence of PE events shows disagreement among different studies, reporting no association⁹ or high risk of recurrence with Odd Ratios (OR) from 2.4 to 41.^{10,11} For FII G20210A mutation the recurrence rates according to Margaglione study is 20%.¹⁰ The risk of recurrence varies with time after the incident event, being highest during the first 6 to 12 months and never falling to zero.¹²

Because of these conflicted results, there is no accepted opinion on DNA testing in patients who develop first episode of DVT or pulmonary embolism as part of prevention strategy of recurrent episodes of VTE.

The aim of this study is to identify the recurrence rate of PE during the first 6 months after the diagnosis while they are on anticoagulation therapy, and also to study the impact of hereditary risk factors on the recurrent rate in Jordanian patients with acute pulmonary embolism during the same period.

Design and Methods

Patients and Laboratory Methods

Free protein S, Protein C, and antithrombin III activity levels were assessed for each plasma sample using commercially available kits LIAtest free Protei S, STACHROM Protein C and STACHROM A III (Diagnostica Stago.France) and fully automated system (STACompact system, Diagnostica Stago. France). Manufacturer instructions were followed.

DNA Isolation

Genomic DNA was isolated from peripheral blood lymphocytes using Wizard DNA purification kit (Promega, USA).

FVL and Prothrombin G20210A Polymorphism

A multiplex Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was used for the simultaneous detection of FVL and prothrombin G20210A mutations. Conditions for multiplex reaction were described by Huber et al.¹⁴ The bands before restriction were 241 bp for factor V gene and a 506 bp for prothrombin G20210A. The PCR products were then digested with HindIII enzyme (Promega, USA) then Digested PCR products were separated on a 3% agarose gel and stained with ethidium bromide. Digestion of the amplicons with HindIII restriction enzyme were as follows: factor V wild-type yielded a 241 bp fragment, factor II wild-type gave 407 bp and 99 bp fragments, FVL heterozygous resulted in 241, 209, and 32 bp fragments, prothrombin G20210A heterozygous yielded 407, 384, 99, and 23 bp fragments, Factor V homozygous yielded 209 and 32 bp fragments and prothrombin G20210A homozygous yielded 384, 99, and 23 bp fragments.

MTHFR Polymorphism

Genotyping was performed for the polymorphisms C677T. Amplification of the C677T region was performed using the forward primer: TGAAGGAGAAGGTGTCTGCGGGA and the reverse primer: AGGACGGTGCGGTGAGAGTG yielding a 198 bp band. The PCR conditions were described by Yi et al.¹⁵ The PCR products of C677T digested with HinfI enzyme (Promega, USA). Resulting fragment were visualized using ethidium bromide staining and 3% agarose (Promega, USA) gel electrophoresis. The digestion fragment sizes for C677T genotypes were: a single 198 bp band for CC, 198, 175 and 23 bp for CT, and 175 bp and 23 bp for TT .

Statistical Analysis

The data were analyzed using SPSS version 16.0 and Epi info version 3.3.2 (2005) software. The Fischer exact test 1 tailed p-value was used for statistical analysis, with a significance level of 5%.

Results

A total of 90 patients were enrolled in this study, only 72 patients, 25 (34.7%) men and 47(65.3%) women with a mean age of 49.97 ±15.4 years who met the inclusion criteria were analyzed. 18 (24.3%) patients were excluded because they lost follow up. 7 out of 72 patients (9.7%) had a recurrent episode of PE within the 6 months of the first episode. 3 out of 7 (42.9%) were men and 4 (57.1%) were women. The patient's characteristics which include gender, age, INR>2, positive anticardiolipin, presence and absence of obvious risk factors are comparable in both groups (those with recurrence and those without recurrence) (Table1). The frequency of protein S deficiency, anti thrombin III deficiency was almost similar in patients who has recurrence

and those who have no recurrence 42.9% vs. 43.1%, p= 0.65. Recurrence of PE did not show any significant difference related to FVL, FII and MTHFR with p-values of, 0.52, 0.73 and 0.34, respectively. There was a significant correlation between the recurrence rate of PE and protein C deficiency p = 0.02 (Table 2).

Discussion

The results of our study showed that the recurrence rate of pulmonary embolism during the first 6 months after the diagnosis of the first episode was 9.7%. The recurrence of PE in our study was statistically higher in patients with protein C deficiency. The presence of other inherited thrombophilic risk factor according to our study didn't show increased incidence of recurrence of PE after the first episode.

The previous studies showed conflicting results regarding the effect of inherited thrombophilic factors on the recurrence rate of PE. Brouwer et al. found that patients with a hereditary protein S, protein C or antithrombin deficiency appear to have a high absolute risk of recurrence VTE.¹⁶

Table (1): Patients' Characteristics with or without recurrent PE regarding the age, gender, and acquired risk factors for PE.

Patients Characteristics	Total No= 72	Recurrent PE No= 7 (9.7%)	No Recurrent PE No =65 (90.3%)	P-value
Mean Age ± SD	49.97 ± 15.4	46.86 ± 18.48	50.31 ± 15.18	
Range	17-80 years	17 -75 years	17 – 80 years	
Male	25(34.7 %)	3 (42.9%)	22 (33.8%)	0.463
Female	47(65.3%)	4 (57.1%)	43 (66.2%)	
Age < 45	30 (41.7%)	4(57.1%)	26 (40.0%)	0.315
INR > 2.0	40 (55.5%)	3 (42.9%)	37 (56.9%)	0.374
Obvious risk factors	49 (68.1%)	4 (57.1%)	45 (69.2%)	
No obvious risk factors	23(31.9%)	3 (42.9%)	20 (30.8%)	0.396
Phospholipids deficiency	3 (4.2%)	1(14.3%)	2(3.1%)	0.732

* INR International Normalized Ratio

Table (2): Frequency of inherited risk factors among patients with or without recurrent PE.

Thrombophilic Factors	Total No=72	Recurrent PE No= 7 (9.7%)	No Recurrent PE No=65 (90.3%)	P-value	OR 95% CI
Protein C deficiency	22(30.6 %)	5 (71.4%)	17(26.2%)	0.025	7.06 (1.06 - 58.79)
Protein S deficiency	31(43.1 %)	3 (42.9%)	28(43.1%)	0.655	0.99 (0.16 - 5.86)
Factor V deficiency	17(23.6 %)	2 (28.6%)	15(23.1%)	0.528	1.33 (0.60 - 9.12)
Factor II deficiency	3(4.2 %)	0 (0%)	3(4.6%)	0.732	0.00 (0.00 - 25.47)
MTHFR deficiency	41(56.9 %)	3 (42.9%)	38(58.5%)	0.345	0.53 (0.09 - 3.15)
ATIII deficiency	0(0 %)	0 (0 %)	0 (0 %)	---	

Heit et al. found an overall cumulative percentage of VTE recurrence at 180 days, 1 year and 10 years of 10.1%, 12.9%, and 30.4%, respectively.¹⁷ Several studies and a meta-analysis have revealed that men have higher risk of recurrence than women.¹⁸ Our study showed that males have higher recurrence but this was not statistically significant, 3 out of 25 (12%) for men vs. 4 out of 47 (8.5%) for women, p value was 0.46. Ho et al. found that Factor V Leiden and the prothrombin G20210A variation, the commonest inherited risk factors for thrombosis, are associated with an increased risk of VTE recurrence.¹⁹ However, the magnitude of the risk conferred by the presence of one of these risk factors is modest and not sufficient to warrant long-term anticoagulation. Baglin et al. and Christiansen et al. found that the risk of recurrence in patients with first VTE and a thrombophilic defect and in patients without a thrombophilic defect was similar.^{20,21}

Different results among the studies may be due to differences in inclusion and exclusion criteria. Therefore, even if not conclusive, the results from our study and the other studies necessitate the need to test patients with unprovoked vein thrombosis for the presence of deficiencies of natural inhibitors in concomitance with other potential risk factors for recurrence of VTE and to individualize future preventive strategies in each patient. Further studies on a national level and including a larger number of patients are needed.

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The Effect of Genetically Related Risk Factors on the Recurrence Rate of Acute Pulmonary Embolism... Nathir M. Obeidat

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أثر عوامل الخطورة ذات العلاقة بالوراثة على معدل تكرار حدوث الانسداد الرئوي الحاد في مستشفى تعليمي في الأردن

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الملخص

الهدف: التعرف على معدل تكرار حدوث الانسداد الرئوي الحاد خلال الأشهر الستة الأولى من تشخيص الإصابة لدى المرضى الذين يخضعون لعلاج منع تخثر الدم، وذلك من أجل دراسة أثر عوامل الخطورة الوراثية على معدل تكرار الإصابة لدى المرضى خلال الفترة ذاتها. **الطرق:** تم إجراء دراسة في مستشفى الجامعة الأردنية، في الفترة الواقعة بين كانون الثاني عام 2005 ونهاية كانون الأول عام 2007. ثم جرت متابعة استمرت حتى تموز 2008. تم فحص (90) مريضاً من المصابين بالانسداد الرئوي الحاد. وقد تم شمول (72) مريضاً منهم فقط بهذه الدراسة، وذلك بسبب فقدان المتابعة للمرضى الباقين.

تم فحص جميع المرضى للوقوف على العوامل المتعلقة بالوراثة (MTHFR, FII, FVL)، ومستوى بروتين (C) الحر في البلازما، وبروتين (S)، والانتثرومبين (III). وتم تقسيم المرضى الى مجموعتين: المجموعة الأولى ضمت المرضى الذين حدث لديهم تكرار للإصابة، والمجموعة الثانية ضمت المرضى الذين لم يحدث لديهم مثل هذا التكرار.

النتائج: تكررت الإصابة لدى (7) من المرضى الـ (72) الذين انطبقت عليهم المعايير (9.7%) خلال الأشهر الستة الأولى من التشخيص. وكانت هناك علاقة ارتباط دالة بين معدل تكرار حدوث الإصابة ونقص بروتين (C) عند مستوى الدلالة (0.025). ولم يكن هناك ارتباط دال بين معدل تكرار حدوث الإصابة وبقية العوامل الوراثية المدروسة.

الاستنتاج: تظهر نتائج هذه الدراسة الحاجة إلى فحص المرضى المصابين بخثار الأوردة للتحقق من وجود نقص في مضادات التخثر الطبيعية، وبخاصة مستوى بروتين (C). أما عوامل الخطورة الأخرى لتكرار الإصابة بالجلطة الوريدية (VTE) فيجب دراستها كل حالة على حدة. هذا بالإضافة إلى الحاجة إلى دراسات أخرى تشمل أعداداً أكبر من المرضى لتوضيح أهمية عوامل الخطورة المذكورة في تكرار الإصابة بالمرض.

الكلمات الدالة: عوامل الخطورة الوراثية، تكرار حدوث الإصابة، الانسداد الرئوي الحاد.