Thrombophilia-related genetic variations in patients with pulmonary embolism in the main Teaching Hospital in Jordan

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ABSTRACT

الأهداف: دراسة تكرار تغيرات العوامل الوراثية لتخثر الدم وهي عامل ليدن الخامس (FVL) والعامل الثاني G20210A FII وعامل مختزلة الميثيلين تترا هيدروفولاتC677T MTHFR لدى مرضى الانسداد الرئوي الحاد. ومعرفة فيما إذا كان تكرار هذه العوامل لدى مرضى الانسداد الرئوي الذين ليس لديهم عوامل خطره ظاهرة لانسداد تجلطي وريدي أكثر منه لدى المرضى الذين لديهم عوامل خطره ظاهرة.

الطريقة: تم إجراء دراسة الحالات في مستشفى الجامعة الأردنية ما بين الفتره 2005 حتى2007. كان عدد مرضى الانسداد الرئوي الحاد 92 مقارنة مع 99 حالة عادية وتم إجراء فحص عوامل تخثرالدم الوراثي لجميع الحالات.

النتائج: أن تكرار التغيرات الوراثية لدى مرضى الانسداد الرئوي هي (23.9%) 22/92 لعامل ليدن الخامس، (3.3%) 3/92 لعامل FII و (52.2%) 48/92 لعامل (MTHFR). ولدى مجموعة التحكم (12.1%) 12/99 لعامل (FVL)، و(0%) 0/99 لعامل (FII) و(53.5%) 53/99 لعامل (MTHFR). كان هنالك دلالة إحصائية بين المرضى ومجموعة التحكم لدى FVL p=0.03. بينما لا يوجد أي دلالة إحصائية ل FII p=0.10 و عامل MTHFR p=0.85. وكان تكرار التغيرات لدى المرضى الذين لا يوجد لديهم عوامل خطره ظاهرة(27.6%) 8/29 لعامل ليدن الخامس، (6.9%) 2/29 لعامل FII، و MTHFR 48.3% 14/29، والمرضى الذين يوجد لديهم عوامل خطره ظاهرة (22.2%) 14/63لعامل ليدن الخامس، (1.6%) 1/63 لعامل (FII) و (52.3%) 33/63 لعامل (MTHFR).

خاتمة: كان عامل (FVL) أكثر لدى مرضى الانسداد الرئوي مقارنة بمجموعة التحكم، وأما تكرار هذه التغيرات لدى العامل (FVL) وعامل (FII) وعامل (MTHFR) لم يكن أكثر لدى مرضى الانسداد الرئوي اللذين ليس لديهم عوامل خطره ظاهرة لانسداد تجلطي وريدي مقارنة مع المرضى الذين لديهم عوامل خطره.

Objectives: To study the frequency of Factor V Leiden (FVL), prothrombin gene mutation G20210A and methylenetetrahydrofolate reductase C677T in patients with acute pulmonary embolism (PE); and to investigate whether these factors are more frequent in patients who have no obvious risk factors for venous thrombo-embolism compared to those with obvious risk factors.

Methods: A case-control study conducted at Jordan University Hospital, Amman, Jordan during the period 2005-2007. Compared 92 patients with acute PE to 99 normal subjects. All subjects were investigated for the 3 genetically related thrombophilic factors.

Results: The frequency of these factors in patients were 22/92 (23.9%) FVL, 3/92 (3.3%) Factor II (FII) and 48/92 (52.2%) methylenetetrahydrofolate reductase (MTHFR). In the control group, FVL was 12/99 (12.1%), FII 0/99 (0%), and 53/99 (53.5%) MTHFR. There was a statistically significant difference between patients and controls for FVL (p=0.03), but no statistical significance for FII (p=0.10) and MTHFR (p=0.85). In patients with no obvious risk factors, the frequency of these factors were 8/29 (27.6%) FVL, 2/29 (6.9%) FII, and 14/29 (48.3%) for MTHFR compared to patients with obvious risk factors 14/63 (22.2%) for FVL, 1/63 (1.6%) FII, and 33/63 (52.3%) MTHFR.

Conclusion: The FVL is statistically more frequent in patients with PE compared to the control group, and the frequency of FVL, FII, and MTHFR is not significantly higher in patients with acute PE who have no obvious risk factors compared to those with obvious risk factors.

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Pulmonary embolism (PE) is a common clinical disorder that is associated with high morbidity and mortality if untreated.[1,2] Considerations of risk factor assessment needs a structured protocol to facilitate early diagnosis of PE, and to ensure early and adequate treatment.[3] The risk factors for PE can be acquired or genetically related. Both play an important role in the development of venous thromboembolism (VTE).[4-6] Patients who develop acute PE may or may not have obvious risk factors for VTE. Large population-based studies have found that 25-50% of patients with confirmed PE,[7] have no identifiable risk factors for venous thrombosis including cancer, antecedent trauma, recent surgery or immobilization.[4,7-10] The most important thrombophilia related genetic factors are factor V Leiden (FVL), the factor II (FII; prothrombin G20210A), and methylenetetrahydrofolate reductase (MTHFR) C677T.[7,11,12] The aim of this study is to measure the frequency of the thrombophilic genotypes FVL, prothrombin G20210A, or MTHFR C677T in patients diagnosed as acute PE, and to determine whether these risk factors are more frequent in patients who developed PE without having obvious risk factors for VTE.

Methods. A case-control study was conducted at Jordan University Hospital, Amman, Jordan during the period 2005-2007. A total of 92 Jordanian patients (34 men and 58 women) with a confirmed diagnosis of PE were included. The characteristics of these patients are showed in **Table 1**. In patients with clinical suspicion of PE, the diagnosis was confirmed radiologically by an independent radiologist. The radiological diagnostic studies, which were carried out included spiral computed tomography (CT) with PE protocol, and lung perfusion scan with chest x-ray carried out at the same time.[13] In some cases, pulmonary angiogram also had to be carried out. The control group consisted of 99 unrelated healthy individuals with no history of myocardial infarction, deep vein thrombosis (DVT), PE, or stroke. These individuals are either from the hospital personnel, or workers in the faculty of medicine, and small number are visitors to the hospital. Prothrombotic determinants, namely FVL (1691GA), FII prothrombin gene mutation (20210GA), and MTHFR mutation (677CT) were studied in patients and control subjects. The patients were divided into 2 groups, one includes those who have no obvious risk factors for VTE and second group includes those who have obvious risk factors for VTE. The patients were classified in the first group if they did not have any of the following risk factors: Age more than 60 years, pregnancy, or postpartum (<4 weeks) status, use of any drug containing exogenous estrogen or estrogenic drug treatment, congestive heart failure, any history of malignancy, connective tissue disease, or, any recent surgery, limb immobilization >48 hours, indwelling central venous catheter, previous VTE, family history of VTE, or a body mass index >40 kg/m^2.[14,15] The second group comprised patients who have PE with one or more of the risk factors mentioned above. The study protocol was approved by the Independent Ethics Committee at the Faculty of Medicine. All investigated subjects gave written informed consent in accordance with the Helsinki declaration.

Sample collection and DNA analysis. Polymerase chain reaction–restriction fragment length polymorphism of FII, FVL and MTHFR (C677T). For genetic testing, we took a sample of 4.5 ml, 0.5M ethylenediaminetetraacetic acid-anticoagulated blood. The DNA was extracted using Promega DNA (Purification Kit, Madison, Wisconsin (WI), USA) according to manufacturer's instructions. For FII and V, 500 ng of genomic DNA was amplified by multiplex polymerase chain reaction (PCR) according to Huber et al,[16] then the PCR product was digested with HindIII (Promega; Madison, Wisconsin (WI), USA), overnight at 37˚C. Digestion of the amplicons with HindIII restriction enzyme were as follow: factor V wild-type yielded a 241 bp fragment, FII 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. For MTHFR 677, 400 ng of genomic DNA was amplified by PCR according to Yi et al,[17] then the PCR product was digested by Hinfl (Promega; Madison, Wisconsin (WI), USA) overnight

Table 1 - Baselines and clinical characteristics of cases and control subjects.

Age and gender distribution	Patients (PE) n=92	Control n=99
Men	34 (37%)	38 (38.4%)
Mean age in men (years)	46.95	30.1
Women	58 (63%)	61 (61.6%)
Mean age in women (years)	51.05	31.3
Age in years (mean ±SD)	49.54 ± 16.69	31.04 ±10.11
Age range in years	15-85	17-58

PE - Pulmonary embolism

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at 37˚C. All PCR products were analyzed in 3% agarose gel. 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. We used a case-control design to allow quantitative comparison between the 2 groups. All statistical tests were independent t-test and Pearson Chi square was used to test for association in the distribution of categorical variables thrombophilia genetic factors (FVL, FII, MTHFR). The *p*-value less than ∝ level of 0.05 was considered statistically significant. Because of the small number in some of the cells (FII in control subjects was not detected), Fisher's exact test was used to assess the statistical significance of the association. The analyses were performed using the SPSS package version 16.0.

Results. ***Clinical characteristics.*** Between 2005 and 2007 we enrolled 92 patients diagnosed with acute PE. The patients' characteristics are shown in **Table 1**. The number of patients with obvious risk factors was 63 (68.5%), 21 (33.3%) are men and 42 (66.7%) are women. The number of patients without risk factors was 29 (31.5%), 13 (44.8%) are men and 16 (55.2%) are women. Our results also showed no statistical difference between the patients with obvious risk factors and those without risk factors regarding the mean age, and body mass index (**Table 2**).

Factor V Leiden. Among all patients diagnosed with PE, 23.9% had FVL, 21.7% were heterozygous and 2.2% were homozygous, which is statistically significantly different from the frequency of FVL in the control group (12.1%) (**Table 3**). The frequency of FVL in patients with PE and no obvious risk factor was 27.6% while in patients with PE with obvious risk factors it was 22.2% (**Table 4**).

Factor II G20210A. The frequency of FII G20210A in patients with PE was 3.3%, while in the control group the frequency of FII G20210A was 0% (**Table 3**). The frequency of FII G20210A in-patients with or without risk factors was statistically not significant. (**Table 4**).

MTHFR C677T. The frequency of MTHFR, C677T variant was 52.2% in the PE group compared to 53.5% for the control group, this difference is statistically not significant (**Table 3**). The frequency of a MTHFR, C677T variant in patients who have no obvious risk factor was 48.3%, while in patients with PE who have obvious risk factors for VTE the frequency of PE was 52.4%, this difference is statistically not significant (**Table 4**).

Table 2 - Patients' characteristics among the patients with obvious risk factors for VTE and for patients without obvious risk factors.

Patients characteristics	Patients with obvious risk factors n=63 (68.5%)	Patients with no obvious factors n=29 (31.5%)	*P*-value
Age (Mean ± SD)	49.7±17.3	49.1±15.6	0.32
BMI Kg/m^2 (Mean ± SD)	30.03±6.6	27±4.85	0.11
Men	21 (33.3%)	13 (44.8%)	0.289

BMI - body Mass index, VTE - venous thromboembolism

Table 3 - Frequency of genetically related thrombophilic factors in all patients and control group. n(%)

Thrombophilic factors	Cases n=92	Control n=99	*P*-value
FVL	22/92 (23.9)	12/99 (12.1)	0.03
Heterozygous	20/92 (21.7)	12/99 (12.1)	
Homozygous	2/92 (2.2)	0/99 (0)	
FII G20210A	3/92 (3.3)	0/99 (0)	0.10
MTHFR C677T	48/92 (52.2)	53/99 (53.5)	0.85

FVL - Factor V leiden, FII - factor II, MTHFR - methylenetetrahydrofolate reductase

Table 4 - Frequency of genetically related thrombophilic factors among both groups (patients with obvious risk factors for VTE and the group without obvious risk factors for VTE) n(%)

Thrombophoilic factors	Patients with obvious risk factors n=63	Patients without obvious risk factors n=29	*P*-value
FVL	14 (22.2)	8 (27.6)	0.45
FII G20210A	1 (1.6)	2 (6.9)	0.23
MTHFR C677T	33 (52.4)	14 (48.3)	0.78

FVL - Factor V leiden, FII - factor II, MTHFR - methylenetetrahydrofolate reductase, VTE - venous thromboembolism

Discussion. Genetic thrombophilic factors and its role in the development of VTE have been increasingly recognized.[3] We studied these thrombophilia-related genetic variations in patients with PE regardless whether they have, or have no obvious risk factors for VTE.

The results of our study showed that FVL is more common in patients with PE compared to the control group. The FII G20210A was higher in the PE group compared to control group, but this is not statistically significant, and MTHFR C677T was not higher among the patient's group compared to the control group. These results are similar to the results found by Ivanov et al,[4] who studied the impact of thrombophilic genetic factors on PE in 51 patients, and found that FVL was positive in 23.5% of the patients versus 7.1%

of the control group (p=0.01), and FII G20210A was found in 5.9% versus 2% in the control group p-value was not significant.[4] The prevalence of FVL, prothrombin G20210A, and MTHFR G677A among 594 Jordanian patients with both arterial and venous thrombosis was studied by Eid et al,[18] they found FVL positive in 25.7% (20.7% heterozygous, 5% homozygous), prothrombin G20210A mutation in 6% of patients (5.8% heterozygous, 0.2% homozygous), and MTHFR G677A mutation in 31.7% of patients (25% heterozygous, 6.7% homozygous). Eid's study concluded that FVL, and prothrombin G20210A are common among patients with venous thrombosis while, the MTHFR C677T mutation is common in arterial thrombosis.[18] In their study, there was only one out of 30 (3.3%) patients with PE that had a mutation of FII, which is in agreement with our results.[18] Obviously Ivanov[4] and Eid's[18] studies showed that FVL is more common in patients with VTE, and these results agree with our findings that FVL is more common in patients with PE, taking into consideration that our study has larger number of patients with PE. Pathare et al[19] studied 39 Omani patients with VTE, only 8 patients had PE and he found that FVL, and the prothrombin gene mutation (20210G) could not be documented in any of his patients studied. None of the ethnic Omani blood donor controls (n=80) showed mutations in FII and factor V, while MTHFR mutation (C677T) was seen in 14/39 patients (36%).[19] These results from the Omani study, disagree with the results of our study, and both Eidd's[18] and Ivanov[4] studies', taking into consideration that the Omani study includes only 8 patients have PE and the zero incidence of this mutation among the Omani control group.

Our study showed higher frequency of FVL and prothrombin gene mutation (20210G) among PE patients who have no obvious risk factors for VTE compared to those with obvious risk factors for VTE. The frequency of FVL was 27.6% in patients without obvious risk factors for VTE compared to 22.2% among patients with obvious risk factors. The frequency of FII was 1.6% in our PE patients with obvious risk factors for VTE, compared to 6.9% in those patients without obvious risk factors. The frequency of MTHFR C677T was 52.4% in our patients with obvious risk factors for VTE compared to 48.3% in those patients without obvious risk factors. These results are comparable to the results from Kruse et al[7] who found that the frequency of either the FVL or prothrombin sequence variant was not increased in idiopathic PE patients compared with non idiopathic PE patients, they found that 5 of 49 (10%; 95% confidence interval (CI): 0.03-0.22) patients with idiopathic PE had either the FVL or prothrombin sequence variant, compared with 21 of 152 (13%; 95% CI: 0.08-0.20) of non idiopathic PE.[7] Therefore, our study found that the mutations in FII and factor V are higher in patients with acute PE with no obvious risk factors for VTE, but without statistical significance.

Our study included only patients with PE, and did not include other locations of VTE, like DVT in the lower limbs or in upper limbs, and renal vein thrombosis and so forth. So, we could not assess the effect of the site of VTE on the frequency of the thrombophilia-related genetic variations, which was shown to be of importance by Martinelli I,[20] who found that FV Leiden is more prevalent in patients with DVT than in those with isolated PE.

We conclude that FVL is a significant risk factor for acute PE, and the MTHFR mutation is not more frequent in Jordanian patients with PE compared to the control group, while the FII mutation is more frequent in the patent's group, but without statistical significance. Although FVL and mutations in FII are higher in patients with acute PE who have no obvious risk factors for VTE compared to those with obvious risk factors, this difference is not statistically significant.

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