

Frequency of Factor II G20210A and Factor V Leiden Mutations in Algerian Patients with Venous Thromboembolism

Chalal N¹, Demmouche A^{1*} and Cherif touil S²

¹Department of Biology, Faculty of Natural science and life, Djillali Liabes University of Sidi Bel Abbes, Algeria

²Medical laboratory of Sig. Mascara, Algeria

*Corresponding author: Abbassia Demmouche, Doctor in Biology at Djillali Liabes University of Sidi Bel Abbes, Algeria, Tel: +213 773620637; E-mail: demmoucheabbassia@yahoo.fr

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Abstract

Several inherited polymorphisms are associated with the risk of venous thrombosis, including mutation at codon 506 of the factor V gene and mutation at position 20210 of the prothrombin gene. The aim of this study was to determine the frequency of factor II G20210A and factor V Leiden mutations in Algerian patients with venous thromboembolism disease.

In this study, genotyping for factor V Leiden and factor II G20210A mutations was performed in 40 patients with venous thrombosis by the GeneXpert Dx.

According to the results of the study, factor V Leiden was detected in 5 patients (12.5%) and factor II G20210A was detected in only one patient (2.5%). Both mutations were found in heterozygous form and no patient was double heterozygous.

In conclusion, the prevalence of factor V Leiden mutation was high among Algerian patients with venous thromboembolism disease however, further studies are needed in order to estimate the true prevalence of factor II G20210A and factor V Leiden mutations within this population as well as to verify where exactly they have occurred first and how they were carried to other parts of the world. Furthermore, it is hoped that new approaches namely the GWAS could be adopted towards the identification of new genetic risk factors within the Algerian population.

Keywords: Venous thrombosis; Factor V Leiden; Factor II G20210A; Algeria

Introduction

Venous thromboembolism (VTE), whose main clinical presentations include deep vein thrombosis (DVT) and pulmonary embolism (PE), represents a major health problem worldwide. It is the third most frequent cause of morbidity and mortality in civilized countries [1-3].

Early epidemiological studies have suggested that the directly standardized incidence of all VTE events is significantly higher among African Americans (138–141 cases per 100,000 individuals per year) than among Caucasians (80–117 cases per 100,000 individuals per year), significantly lower among Hispanic populations (55–61.5 cases per 100,000 individuals per year), and strikingly lower among Asians and Pacific Islanders (21–29 cases per 100,000 individuals per year); [4,5].

Venous thromboembolism is a multicausal disease occurring as the result of interacting genetic, environmental and behavioral risk factors. Some environmental risk factors for venous thrombosis have been known for centuries; they include bed rest, surgery, trauma, plaster casts, pregnancy, puerperium, lupus anticoagulants, cancer and female hormones. Acquired risk factors still play a major role in the burden of venous thrombosis, even though their impact has lessened because of

the implementation of prophylactic anti-thrombotic strategies [6-8]. As for genetic risk factors of VTE, they are subdivided in to those that are strong, moderate and weak. Strong risk factors are deficiencies of antithrombin, protein C and protein S whereas moderately strong are factor V Leiden, prothrombin 20210A, non-O blood group and fibrinogen 10034T. Finally, there are many weak genetic risk factors, including fibrinogen, factor XIII and factor XI variants [9].

According to several epidemiological studies, factor V Leiden and factor II G20210A mutations are weaker risk factors than deficiencies of other natural anticoagulants, and are the most frequent prothrombotic genetic abnormalities leading to thrombophilia however; the prevalence rate of these mutations varies according to ethnic and geographic distribution of the populations [10].

According to literature data, the relative risk of thrombosis is two times higher in patients with the prothrombin gene mutation, three times higher in patients with the mutation for FV Leiden and six times higher in patients who are carriers of both mutations, compared to patients who have the wild-type genotype for both genes. The incidence of venous thromboembolism is lower in prothrombin mutation carriers compared to factor V Leiden mutation carriers [11].

Several studies were conducted to identify the frequency of factor II G20210A and factor V Leiden mutations among VTE patients however, till now and to the best of my knowledge there is no research study performed within this area previously in the west of Algeria.

Thus, the present study is important because this is the first study of its kind in western Algeria.

The aim of this study was to reveal the frequency of factor II G20210A and factor V Leiden mutations in a cohort of adult patients with venous thromboembolism.

Patients and Methods

Screening for factor II G20210A and factor V Leiden mutations was performed in 40 patients hospitalized for venous thromboembolism disease (DVT and /or PE) at the cardiology departments of Sidi Bel Abbes and Tlemcen University Hospital Centers, from January to September 2014. The diagnosis of VTE was confirmed by Doppler ultrasonography or computed tomographic pulmonary angiography.

Demographic characteristics (age, gender) and known risk factors for the thromboembolic disease were collected for all participants of the study.

All patients were fully informed about the study protocol by the main investigator and have consented to participate in the study by signing the written consent. The study was approved by the Ethics Committee of both hospitals.

Screening for factor II G20210A and factor V Leiden mutations was performed in all VTE patients using Cepheid GeneXpert Dx. This System automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in whole blood using real-time Polymerase Chain Reaction (PCR) assays. The system consists of an instrument, personal computer, handheld barcode scanner, and preloaded software for running tests and viewing the results. This system requires the use of single-use disposable cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross contamination between samples is eliminated. The Xpert Factor II & Factor V Assay includes reagents for the detection of Factor II and Factor V normal and mutant alleles from sodium citrate or EDTA anticoagulated whole blood. Each assay cartridge also contains a Probe Check Control (PCC) that verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability. The primers and probes in the Xpert Factor II & Factor V Assay determine the genotype of the Factor II gene (at position 20210) and/or the Factor V gene (at position 1691).

Results

Factor V Leiden and factor II G20210A mutations were investigated in 40 VTE patients including 34 women and 6 men with an age range between 27 and 75 years (mean age: 49 ± 15.6 years).

Overall, 5 out of 40 patients had factor V Leiden mutation and were all heterozygous whereas factor II G20210A mutation was detected in heterozygous form in only one patient. No patient was double heterozygous (Tables 1 and 2).

As for carriers of factor V Leiden mutation, the first patient was a 50-year-old man with PE and had a family history of VTE. The second patient was a 39-year-old woman with both PE and left iliofemoro-popliteal DVT with no apparent clinical risk factor. The other patient, was a 37 year old woman with right femoro-popliteal DVT in the post-partum. Concerning the fourth patient, was a woman aged 45 who had left femoro-popliteal DVT with a family history of VTE. In addition to this, factor V Leiden mutation was also identified in a 43 year old woman with recurrent popliteal DVT of the left leg.

On the other hand, factor II G20210A mutation was detected in heterozygous form in only one patient, a man aged 50 with idiopathic left iliofemoro-popliteal DVT.

	Female (n=34)	Male(n=6)
Factor V Leiden		
Normal	30 (88.23%)	5(83.33%)
Heterozygous	4 (11.76%)	1(16.66%)
Homozygous	0	0
factor II G20210A		
Normal	34(100%)	5(83.33%)
Heterozygous	0	1(16.66%)
Homozygous	0	0

Table 1: Distributions of factor V Leiden and factor II G20210A mutations.

Risk factors	Patients screened for factor V Leiden and factor II G20210A mutations (n=40)	Patients carriers of factor V Leiden or factor II G20210A mutation (n=6)
Age(mean ± SD)	49 ± 15.6 years	44 ± 5.4 years
Immobility	15(37.5%)	0
Pregnancy/postpartum	7(17.5%)	1(16.66%)
Oral contraceptives	4(10%)	0
Obesity	2(5%)	0
Recurrent DVT	2(5%)	1(16.66%)
Spontaneous DVT	3(10%)	2(33.33%)
Family history of DVT	2(5%)	2(33.33%)
Diabetes	3(7.5%)	0
hypertension	3(7.5%)	0
Surgery	8(20%)	0
fractures	4(10%)	0

Table 2: Clinical risk factors in all VTE patients included in the study.

Discussion

Thrombosis occurs as a result of disruptions in the blood clotting system. Therefore, it is crucial to evaluate hereditary and acquired thrombotic risk factors, which may lead to prothrombotic pathological changes in normal coagulation systems [12].

In this study, we investigated factor V Leiden and factor II G20210A mutations in Algerian patients with venous thromboembolism and to our knowledge this is the first study conducted in the west of Algeria.

Among the inherited thrombophilia, factor V-Leiden gene mutation is the most common predisposing factor, accounting for 10% to 20% of VTE in large population studies [13,14]. Some authors suggest that this mutation is present in up to 37% of patients with post thrombotic syndrome [15]. The lifetime probability of symptomatic VTE in patients with heterozygous factor V Leiden mutation is approximately 10% [16]. The risk of VTE is increased 3-7-fold in heterozygotes for factor V Leiden and 50- to 100-fold in homozygotes [14,16].

Factor V Leiden is rarely found in African, Australian and South Asian population and generally is more prevalent in northern Europe with a peak of 8% to 15% in Sweden, and lower in southern Europe, where it ranges between 2% and 4%. In the United States, a prevalence between 5% and 8% was reported [17-19]. Its prevalence in Turkey is as high as 10% [20].

Several studies conducted on Arabs and populations living in the Middle East and North Africa (The MENA region), showed a high prevalence of FV Leiden in these populations, who are not usually classified as Caucasians [21]. However, the MENA region is geographically very close to Europe and had witnessed a lot of human movement from and to Europe, and hence such populations are expected to have some Caucasian genes in their DNA. Therefore, the presence of FV Leiden in Arabs and North African populations should not be a surprising upshot.

According to two studies performed previously in Algeria by Chafa O et al., Bourouba R et al. a prevalence of 13.8% was reported among VTE patients whereas the prevalence of this mutation in normal population varied between 1.3-2% [22,23]. Studies conducted in Morocco showed that factor V Leiden mutation was absent among healthy population however; no data was provided concerning its prevalence among VTE patients, while in Tunisia the frequency of this mutation among VTE patients ranges between 20.3-24.6% and 3-13.6% in healthy population [24-35].

In our study, the frequency of FV Leiden mutation in VTE patients is 12.5% (heterozygous) which is considered as a high prevalence compared to many other countries.

The second most important inherited prothrombotic risk factor is FII G20210A polymorphism, its prevalence in the Mediterranean area ranges around 3.0% (95% CI=2.3-3.7%), while in northern Europe the frequency of this mutation in the population is two times smaller (1.7% (95% CI=1.3-2.2%)). The greatest frequency in the world is found in Mexican population, where the prevalence is extremely high (13.5%) [17]. The prevalence of this mutation in the VTE patients is 8%, and even higher prevalence (18%) is found in selected families with thrombosis [36].

High prevalence of Prothrombin G20210A mutation was also reported in populations living close to Europe, namely countries of the Middle East and North Africa. In fact, the prevalence in these countries was very comparable with the prevalence reported in Southern European countries. Therefore, the countries present on the coasts of the Mediterranean Sea, including Southern Europe, may be grouped together sharing the same prevalence of Prothrombin G20210A mutation. These countries, 20 in total, have a prevalence of 3-24% in patients with VTE and 1-12% in the general population [37].

In Algeria, a prevalence of 6% was reported in VTE patients and 1.8% in healthy population whereas in Morocco, the prevalence of this mutation ranges between 2.4% and 5.5% among healthy population

[24,25,38]. No data could be retrieved from the literature on the prevalence of Prothrombin G20210A in Moroccan VTE patients. In addition to this, a prevalence of 3.2% was found in Tunisian VTE patients whereas its prevalence among healthy population ranges between 0% and 7.4% [26,27,31-33,35,39,40].

Our study showed that only one patient who had factor II G20210A mutation (Heterozygosis). This reflects that prothrombin mutation is less important than factor V Leiden mutation among the Algerian population.

These two mutations interact with other concurrent acquired and thrombophilic conditions such as cancer, surgery, long haul air travel, pregnancy and obesity which increase the risk of incident venous thrombosis. Thus, it is important to emphasize that the presence of polymorphic allele only by itself is not sufficient for the occurrence of thrombosis. All studies conform to the multifactorial etiology of the disease. The risk of the thromboembolic disease increases in proportion to the number of risk factors [9].

There were certain limitations in this study, namely the relatively small sample size used. Thus, further studies are needed in order to confirm the true prevalence of these two mutations among the Algerian population as well as to verify where exactly they have occurred first and how they were carried to other parts of the world.

In addition to the aforementioned, thrombophilia screening should be tailored to accommodate a population's risk factor. In countries with high prevalence of factor V-Leiden or prothrombin G20210A, these mutations should be tested among VTE patients even with a transient risk factor and patients with situations highly suggestive of hypercoagulation states. This permits to extend the duration of anticoagulant therapy in high risk patients reducing the incidence of postthrombotic syndrome and recurrent venous thromboembolism.

Furthermore, it is hoped that new approaches namely the GWAS could be adopted towards the identification of new genetic risk factors within the Algerian population.

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