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Original Article

Prevalence of positive factor V Leiden and prothrombin mutations in samples tested for thrombophilia in Saudi Arabia

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Abstract: Venous thromboembolism (VTE) is a multifactorial disease that results from the interaction of both inherited and acquired risk factors. The complications of these risk factors often lead to significant morbidity and mortality. There are many inherited thrombophilia risk factors, such as factor V Leiden (FVL) and prothrombin gene mutation (PT). The prevalence of these mutations varies among geographical locations and ethnic groups. Objectives: This is a retrospective analysis of laboratory data aimed to estimate the laboratory-based frequency of FVL and PT mutations and assess the concordance between the coagulation assay and FVL molecular test. Methods: The study reviewed the frequency of positive blood samples tested by molecular and functional-based techniques. The demographic and laboratory data of patients tested in molecular and coagulation laboratories at the Institute for Thrombophilia were reviewed and analyzed. Results: A total of 1524 samples were tested for FVL, 1023 for PT, and 1057 for APCR. Results showed that 90 (5.9%) patients were positive for FVL, 30 (2.93%) for PT mutations, and 95 (8.99%) had low APCR, while 38 (3.69%) patients had low APCR with no FVL mutation. Conclusion: This study reports high positive results among patients tested as part of thrombophilia workup or screening for other clinical conditions associated with the increased risk of thrombosis. The limitation of this study was that it had minimal clinical correlation because the data were collected retrospectively from laboratory records.

Keywords: Thrombophilia, F V leiden, prothrombin G20210A, saudi arabia

Introduction

Thrombophilia is defined as the tendency to develop inappropriate blood clots, with venous thromboembolism (VTE) being the most common clinical manifestation of thrombophilia. VTE is considered a major health problem worldwide and is associated with significant mortality and morbidity [1, 2]. There are many risk factors for VTE, which are either inherited, such as genetic predisposition, or acquired, such as surgery, trauma, immobilization, obesity, pregnancy, hormone replacement therapy, use of contraceptives, genetic predisposition, and increased risk of thrombosis [2, 3].

Various genetic defects have been reported to be associated with an increased risk of venous thrombosis. These genetic defects are naturally occurring anticoagulant deficiencies such as antithrombin without (III), protein C, protein S, a polymorphism in methylenetetrahydrofolate reductase (MTHFR) gene, FVL (G1691A), and prothrombin (PT) G20210A mutation [2].

Factor V circulates in the blood in an inactive form which can be activated by thrombin and inactivated by activated protein C through selective proteolytic cleavages at positions Arg 306, Arg 506, and Arg 679. Factor V Leiden mutation (FVL), which is the most common inherited form of thrombophilia, leads to activated protein C resistance (APCR), resulting from the substitution of adenine for guanine at position 1691 of the factor V gene. This causes an amino acid replacement of arginine with glutamine at position 506 in the factor V polypeptide (FV Q506) [1-4].

PT is the inactive precursor of thrombin, which on the other hand, is the end product of the coagulation cascade. PT G20210A mutation,

Table 1. The primers sequences used for FVL and PT mutations detection

Test	Primer sequence
FVL F	GGG CTA ATA GGA CTA CTT CTA ATC TGT AAG A
FVL R	TTC TGA AAG GTT ACT TCA AGG ACA AA
FVL	1691G/A
PMT F	TGT GTT TCT AAA ACT ATG GTT CCC AT
PMT R	CCA TGA ATA GCA CTG GGA GCA T

FVL F (factor V Leiden forward), FVL R (factor V Leiden reverse), PT F (prothrombin mutation forward) and PT R (prothrombin mutation reverse).

characterized by a transition of guanine-to-adenine at position 20210 in the sequence of the 3'-untranslated region of the PT gene, results in the upregulation of PT production, thereby increasing the risk of thrombosis by 2-3 fold [2, 3].

FVL mutation can be detected by testing APCR using a functional clotting-based assay and molecular analysis such as PCR-based assay. The PCR test is not suitable for screening because it is expensive and labor intensive, while the functional assay (APCR test) is highly sensitive (98-100%) and specific (91-98%) for the detection of FVL mutation [1].

This study aimed to estimate the laboratory-based frequency of FVL and PT mutations and study the concordance between the coagulation assay and FVL molecular test.

Materials and methods

This study reviewed the coagulation assay APCR tests and molecular tests for FVL and PT mutations in patients who presented to our institute with VTE or were screened for other clinical conditions associated with increased risk of thrombosis. The institute research committee had approved the study with RAC# 2091056.

Samples with no available demographic like samples from other hospitals sent to our reference lab were excluded.

Demographic and clinical data were collected by reviewing the medical records of all participants sent to the institute laboratory as part of the investigation for thrombophilia. The results for APCR, FVL, and PT mutations were collected and summarized in the form of frequencies and percentages.

Blood samples obtained from January 2013 to December 2018 underwent molecular testing; PT and FVL mutations were analyzed using an SNP Genotyping Assay (a Multiplex end-point Allelic Discrimination Assay). A heterozygous and homozygous wild type/mutant samples were used as controls. The Master Mix set was prepared using a genotyping master mix buffer comprising F-primer, R-primer, wt-probe, mt-probe, and TaqMan reagent. On the instrument, stage 1 was performed at 95°C for 10 minutes, stage 2 was performed at 95°C for 15 seconds, and then at 60°C for 90 seconds. This cycle was repeated 50 times. Allelic discrimination was performed automatically. **Table 1** shows the primers used for both tests.

The samples were tested for APCR using the STA-Staclot APC-R system-STA-R Max Analyzer-STAGO. The APCR was reported as a ratio, with a ratio of <0.86 indicating positive activated protein C resistance [5].

STATISTICS: Frequency and percentage were used to describe data according to different demographic variables. All data were transferred to IBM SPSS Statistics Version 20 (IBM Corp., Armonk, NY, USA) for final analysis.

Results

All samples with results performed between January 2013 to December 2018 were included in the study. There were 1524 participants (337 men and 1187 women). The age of the subjects ranged from 4 months to 69 years with a median age of 32 years. Among 1524 samples tested for FVL, only 1023 were tested for PT and 1057 were tested for APCR. Furthermore, 90 (5.9%) samples were positive for FVL mutation (84 GA heterozygous and 6 AA homozygous) and 30 (2.9%) samples were positive for PT mutation (29 GA heterozygous and 1 AA homozygous) (**Table 2**). The APCR was low in 95 (8.99%) samples, and approximately 30% were positive in males. There was a positive relationship between APCR and FVL mutations, and 38 (3.6%) samples had a low APCR and no FVL mutation, while one patient showed FVL mutation with normal APCR. Further analysis of this acquired APCR were found to be associated with other disorders. The most frequent

Table 2. Data on types of mutations

Variables	N	Positive cases (n)	Positive cases (%)
FVL hetero	1524	84	5.51
FVL homo	1524	6	0.39
PTM hetero	1023	29	2.83
PTM homo	1023	1	0.10

FVL hetero (factor V Leiden heterozygous), FVL homo (factor V Leiden homozygous) PT hetero (prothrombin heterozygous) and PT homo (prothrombin homozygous).

Table 3. The clinical conditions with acquired APCR positive samples

Clinical condition	n	%
APS	22	57.9
CVA	5	13.2
SLE	4	10.5
Hyperhomocysteinemia	3	7.9
ET	2	5.3
HIT	1	2.6
Malignancy	6	15.8
No association	2	5.3
SCD	1	2.6

(APSD) antiphospholipid syndrome, (CVA) thrombotic stroke, (SLE) systemic lupus erythematosus, (ET) essential thrombocytosis, (HIT) heparin-induced thrombocytopenia and (SCD) sickle cell disease.

associated condition was antiphospholipid syndrome were seen in 22 (57.9%) patients while other less common conditions included, 5 (13.2%) patients with thrombotic stroke, 6 (15.8%) patients with different malignancies, 2 (5.26%) patients with essential thrombocytosis, 4 (10.5%) patients with systemic lupus erythematosus, and 3 (7.89%) patients with high homocysteine. One patient was diagnosed with sickle cell disease and one with heparin-induced thrombocytopenia (2.63%) (**Table 3**).

Discussion

Thrombophilia or hypercoagulability is a condition that increases the risk of thrombosis; many acquired and inherited factors are associated with thrombophilia. Venous thromboembolism is an example of a multigenic disorder with interactions between various genetic factors that often synergize to increase the risk of disease [6]. FVL and PT mutations are known prothrombotic factors that have variable risk for thrombosis. Although FVL is considered the most common hereditary thrombophilia, there is variability in the prevalence of these genetic mutations among ethnic populations [7-9]. The mutation is high in Europeans and low among the populations of Southeast Asia and Africa. The reported mean frequency was 2.78%, with a peak value of 12% [10]. The present study showed that 5.9% of samples were positive for FVL (5.5% heterozygous and 0.39% homozygous), a value that is higher than that reported in a previous study conducted by Saour et al. who reported a 1.3% frequency of FVL in healthy individuals [11]. In another study, Almawi et al. reported a 2.0% frequency of G/A genotype among the Saudis [12]. Current frequencies are higher because they have been tested among the high-risk group with clinical presentation of thrombosis. Rola et al. also reported a prevalence of 14.9% of FVL among Saudi women with miscarriage [13].

Arab countries have a central geographical location in the Middle East between Europe (where the prevalence of FVL is very high), Africa, and Asia (where the prevalence is very low) with movements of people between the three continents. Different studies showed heterogeneity in the distribution of FVL mutation among Arabs, ranging from 21.8% in Jordanians to 0% in Moroccans [10-18]. Almawi et al. reported high frequencies of the heterozygous FVL genotypes in Lebanon (13.8%) and Tunisia (5.8%) and low frequencies in Bahrain (3.1%) [12]. A low prevalence was not detected in some Arab populations such as Omani and Moroccan [15, 16]. Dashti et al. confirmed the variations in the prevalence of FVL in different Arab countries, where it was relatively high among Arabs living in the Eastern Mediterranean Sea (Palestinians 25%, Jordanians 23.5%, Syrians 16%, and Egyptians 15%) and relatively low among Arabs who originate from the Arabic peninsula, such as Kuwait (4.5%) [17].

The frequency of FVL mutation in healthy European Caucasian populations ranges from 2.5% to 13.3% with a pooled prevalence of 4.7% in Greece and the highest prevalence in Sweden [6, 7]. The frequency of FVL in people of African descent is estimated to range from 0% to 1.3%) and is reportedly 0.6% in Asia-Minor and 0% in Southeast Asia, America, and Australasia [6, 8, 9, 19, 20]. The frequency of homozygous was much lower than the heterozygous (5.9% and 0.39%, respectively). This

has been shown in many previous reports. Maalej et al. reported that, in South Tunisia, the prevalence of FVL mutation was 13.6% (heterozygous: 12.4%, homozygous: 1.2%) [21]. In addition, Angelopoulouk et al. reported a different prevalence of FVL in a Greek-Cypriot population, where 12.2% were heterozygous and 1.1% were homozygous [22].

The PT G20210A mutation showed less variability among different ethnic groups. Our report showed that 2.9% of people were positive for PT (2.8% heterozygous and 0.1% homozygous), which is similar to reports in other Arab populations such as 3.6% in Lebanese, 2.6% in Tunisian, 2.0% in Jordanian, and 2.7% in Moroccan populations, and higher than that reported in Bahrain (1.0%) and Egypt (1.43%) [12, 15, 23, 24]. Conversely, the current findings are contrary to those of Almawi et al., who reported the absence of PT among Saudi subjects [12]. Pathare et al. also reported the absence of the PT gene mutation among Omani subjects [16]. Almawi et al. reported the prevalence of the heterozygote and homozygous variants for PT mutation among Lebanese to be 19.2% and 3.6%, respectively, while the prevalence among Palestinians is reportedly 9.1% [25, 26].

The distribution of the PT mutations varies within Europe, with a prevalence of 1% to 6% among Caucasian populations, whereas in non-Caucasian populations, it is very rare or absent [27]. PT gene mutation was very rare in individuals from Asian and African countries [28]. The study reported very rare cases of homozygous PT mutation, which were observed in other populations. Western Iran, a neighboring country has a prevalence of PT mutation of 1.6% heterozygous and 0% homozygous [29]. In Caucasian-Australian population, the prevalence of the PT mutation was 4.1% for heterozygous and 0.2% for homozygous mutations, while in the Chinese population, it was very rare or absent [30-32].

The relationship between functional and molecular tests for FVL was previously studied. Although agreement was found in almost 96% of our cases, Clark et al. reported a relatively low concordance around 78.3% in a study with a small sample size. Acquired APCR in the absence of FVL mutation has been reported in a number of conditions including antiphospholipid syndrome and malignancies, as reported in previous studies [33-35].

Conclusion

In conclusion, our findings confirm previous reports regarding the prevalence of FVL and PT mutations in Saudi populations. We demonstrate, in larger patient samples, that there is a higher frequency of both mutations in this population and that the frequency it is similar to that in the Caucasian population.

Disclosure of conflict of interest

None.

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