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Factor V Leiden (G1691A) mutation in Yemeni subjects with thrombophilia

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Abstract

Abstract: Factor V Leiden (G1691A) mutation is a significant biomarker for the evaluation of tendency for venous thrombosis. The objective of our study was to investigate the association between the Factor V Leiden (G1691A) mutation and thrombophilia in Yemeni patients residing in Sana'a City.
Methods: The study included 267 subjects (125 thrombophilia patients as cases, and 142 healthy subjects as controls) who were genotyped using the Single Nucleotide Polymorphism (SNP) Genotyping Assay (FVL Real Time PCR Kit), and the genotypic and allelic frequencies of the factor V Leiden (G1691A) mutation were calculated. The laboratory data of the patients and controls were reviewed and analyzed in Aulaqi specialized medical laboratories.
Results: Factor V Leiden (G1691A) mutation was present in 11.2% of all cases (heterozygotes, GA: 10%, homozygote mutant, AA: 0%) and 1.4% of all control subjects (heterozygotes: 1.4%, homozygote mutant: 0%). The prevalence of heterozygote (GA) genotype for Factor V Leiden (G1691A) mutation was significantly more frequent in the group of subjects with the thrombophilia than in the control healthy subjects, OR (95% CI) = 8.83 (1.97 - 39.66), P= 0.0045). The Factor V Leiden (G1691A) mutation risk (mutant) allele A frequency was significantly associated with thrombophilia [OR (95% CI) = 8.36 (1.01 - 68.96), P = 0.048].
Conclusion From this study, we conclude that the Factor V Leiden (G1691A) mutation is significantly associated with an increased risk of thrombophilia.

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1. INTRODUCTION

Thrombophilia is a pathological condition characterized by aberrant blood coagulation processes that result in an elevated propensity for thrombosis, identified as a state of hypercoagulability. Individuals exhibiting hypercoagulable conditions are predisposed to thrombosis, particularly venous thromboembolic disorders (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE). The incidence of VTE is correlated with significant morbidity and mortality outcomes [1, 2]. A positive familial history of VTE has been recognized as a salient risk factor for the onset of VTE, thereby augmenting the likelihood of inherited thrombophilia [3, 4, 5]. Venous thrombosis risk factors can be systematically categorized into two primary categories: genetic factors, encompassing genetic predisposition, and acquired factors, including surgical interventions, traumatic injuries, prolonged immobilization, obesity, pregnancy, hormone replacement therapy, and the use of contraceptives or heparin [6, 7, 8]. Abnormalities in protein C, protein S, and anti-thrombin III, either quantitative or qualitative, are examples of genetic risk factors. Furthermore, there are additional hereditary thrombophilic disorders characterized by mutations in Factor V Leiden (FVL) (G1691A), prothrombin (PT) (G20210A), and methylene tetrahydrofolate reductase (MTHFR) (C677T), which have been extensively implicated as significant genetic risk factors in people with or without an apparent cause for developing VTE and who have a propensity to recur [8, 9, 10, 11].

The most common genetic cause of inherited VTE is the FVL (G1691A) mutation, which results in a modified form of coagulation factor V that is resistant to inactivation by activated protein C (APC), thus precipitating hypercoagulability or activated protein C resistance (APCR). A high prevalence of the FVL (G1691A) mutation is observed in Caucasian and Arab populations, whereas it is nonexistent in most Asian and African countries [8, 12, 13, 14, 15, 16]. We aimed to investigate the frequency of the FVL (G1691A) mutation in Yemeni patients with thrombophilia and to determine the association between the Factor V Leiden G1691A mutation and thrombophilia risk factors.

2. MATERIALS AND METHODS

In the present case-control study, 125 subjects (53 male and 72 female) who were referred to Aulaqi specialized medical laboratories for thrombophilia testing following VTE or screening for other clinical conditions correlated with an elevated risk of thrombosis from January 2018 to December 2023 were designated as the case group, while 142 healthy subjects (60 males and 82 females) devoid of both personal and familial histories of thromboembolic disorders were included in the control group. The ages of all the participants ranged from 1 to 60 years. The study will be conducted by reviewing the molecular tests for subjects who tested positive for a FVL (G1691A) mutation from our Laboratory Information System. The medical records of the participants sent to the laboratory will be reviewed to gather demographic and clinical data, such as age, sex, laboratory results, and outcomes. Frequencies and percentages were used to compile and summarize the FVL (G1691A) mutation results.

2.1. Molecular testing

Blood samples from all participants were analyzed using a single nucleotide polymorphism (SNP) Genotyping Assay (FVL (G1691A) mutation Real Time PCR Kit, SNP Biotechnology R and D Ltd., Turkey). System conditions including wild-type PCR master mix, mutant type PCR master mix, and DNA control reagents were used for the FVL (G1691A) variant. The real-time PCR kit for the FVL (G1691A) variant provides reagents in a ready to use master mix format that has been specifically optimized for($5^{/}$ nuclease) PCR utilizing the patented SNP analysis method. The test protocol was designed for use with specific primers and probe sequences. Stage 1 of the PCR protocol for each cycle was performed at $95C^0$ for 3 min, followed by stage 2 at $95C^0$ for 15 s and stage 3 at $60C^0$ for 30 s. This cycle was repeated 30 times. Allelic discrimination (genotypic and allelic frequencies of the FVL (G1691A) variant) will be conducted automatically.

2.2. Statistics

Genotypic frequencies and percentages were determined using the SPSS version 26. Allelic frequency was analyzed using descriptive statistics (Allele Frequency Calculator). We assessed the Hardy-Weinberg equilibrium of the genotypic distribution of the FVL (G1691A) variant using the chi-square (χ^2) test (Hardy-Weinberg calculator). To evaluate the effect of this mutation on the risk of thrombosis, statistical results are presented as OR) and 95% CI), and with a P-value < 0.05 was considered significant.

3. RESULTS

A single nucleotide polymorphism SNP genotyping assay was performed to detect the mutations and determine the genotypes of the target genes. The distribution of genotypes for the FVL (G1691A) SNP was consistent with the Hardy-Weinberg equilibrium (P > 0.05). Tables (1 and 2) provide a summary of the prevalence of mutations, genotypes, and allelic frequencies among the study subjects. prevalence of FVL (G1691A) mutations among 125 case subjects and 142 controls was 11.2 % (14 subjects) and 1.4% (2 subjects), respectively, where all had G/A mutant alleles (heterozygote individuals). The mutant (risk) A allele frequencies of FVL (G1691A) were 5.6% and 0.7% in case group and controls, respectively. The risk allele A (mutant) of the Factor V Leiden (G1691A) mutation frequency was significantly associated with thrombophilia $[OR(95\%CI) = 8.36(1.01 - 68.96), P = 0.048]$. The frequency of heterozygote (GA) genotype versus wild (GG) genotype was significantly higher in subjects with thrombophilia when compared to control group (11.2% vs. 88.8% and 1.4 % vs. 98.6 %, respectively), $[OR(95\%CI) = 8.83(1.97 - 39.66), P = 0.0045]$.

Table 1. prevalence of Factor V gene mutation in the studied subjects

Group	Type of Mutation	No. of Subjects (n)	Positive Subjects (n)	Positive Subjects (%)
Case	Factor V Leiden (G1691A) (Heterozygous and Homozygous Mutant)	125	14	11.2
Control	Factor V Leiden (G1691A) (Heterozygous and Homozygous Mutant)	142	2	1.4

Table 2. Genotypic and allelic frequencies observed in the studied subjects

	Case $n = 125$	**Control** $n = 142$	**OR (95% CI)**	$(Pvalue)^a$
	n(%)	n(%)		
Genotypic frequencies				
GG	111 (88.8%)	140 (98.6%)	8.83 $(1.97 - 39.66)^b$	0.0045^*
GA	14 (11.2%)	2 (1.4%)		
AA	0 (0.0%)	0 (0.0%)		
Allelic frequencies				
G (Wild)	236 (94.4%)	283 (99.3%)	8.36 $(1.01 - 68.96)$	0.048^*
A (Mutant)	14 (5.6%)	2 (0.7%)		

Where n is number, OR denotes odds ratio, CI is confidence interval, a represents P-value calculated by odds ratio calculator, b is GA versus GG, and $(*P < 0.05)$, is statistically significant.

4. DISCUSSION

The assessment of genetic risk factors has emerged as a crucial element in the diagnostic evaluation of patients exhibiting clinical signs and symptoms indicative of venous thrombosis [17]. Pulmonary embolism (PE) and deep vein thrombosis (DVT) are distinct manifestations of venous thromboembolism (VTE), a singular pathological condition. The most common VTE disease is DVT [18] . Thrombophilia is a hypercoagulable disorder that increases the risk of developing thrombosis. This can be acquired on a genetic basis. Only reciprocal interactions between genes and the environment can cause clinical manifestations of this multifactorial condition. This condition is the primary cause of venous thromboembolism (VTE) [19, 20]. The prevalence of mutations in thrombophilia-associated genes differs between populations and ethnic groups. The principal genetic factors contributing to inherited thrombophilia and thrombosis include pathogenic variants of the Factor V Leiden (FVL), prothrombin (PT), and Methylene Tetrahydrofolate Reductase (MTHFR) genes [21]. The FVL (G1691A) mutation, regarded as the most significant genetic contributor to inherited VTE, is characterized by a single-nucleotide polymorphism involving the substitution of guanine (G) with adenine (A) at nucleotide position 1691 within exon 10 of the factor V gene. This genetic alteration (FVL mutation) leads to a modified form of coagulation factor V that is resistant to inactivation by activated protein C, consequently inducing a state of hypercoagulability or enhanced susceptibility to thrombosis [22]. In other words, the FVL (G1691A) mutation resulted in a single amino acid alteration (replacement of arginine with glutamine at the 506th amino acid position). This change eliminated the Arg506 cleavage site for activated protein C in Factor V. The presence of the FVL (G1691A) mutation amplifies the risk of thrombosis, as activated protein C, a physiological anticoagulant, is unable to bind and inactivate factor V because the mutation affects the binding domain for activated protein C on factor V. Hence, the active form of factor V is not inactivated, maintaining its activity and further augmenting the risk of thrombosis [23, 24]. The FVL (G1691A) mutation is the most prevalent hereditary risk factor associated with venous thrombosis. This mutation exhibits the highest prevalence of approximately 15% within the European population afflicted with thrombosis, while its occurrence is nearly 5% among white Americans and Canadians [25, 26]. In Asia, the prevalence of this mutation has been documented at 1.9% and 2.5% in the healthy populations in northern India and Saudi Arabia, respectively [27]. The prevalence of thrombophilia resulting from pathogenic variants of the FVL gene among Arab populations has not been adequately studied and explored [15]. Our investigation represents the first inaugural study aimed at evaluating the prevalence of thrombophilia associated with the FVL (G1691A) mutation and the correlation between the Factor V Leiden G1691A mutation and thrombophilia risk factors in Yemen.

The present study revealed that the prevalence of FVL (G1691A) variant is significantly elevated in case subjects (11.2 %) relative to the healthy controls (1.4%), and the occurrence of heterozygote (GA) genotype for FVL (G1691A) mutation was significantly more prevalent in the subjects with the thrombophilia (11.2%) than in the healthy subjects (1.4%), $OR(95\%CI) = 8.83(1.97 - 39.66), P = 0.0045)$. The risk allele A (mutant) of the Factor V Leiden (G1691A) mutation frequency was significantly associated with thrombophilia $[OR(95\%CI) = 8.36(1.01 - 68.96), P = 0.048]$. The findings from this study indicate that the Factor V Leiden (G1691A) mutation is significantly associated with an increased risk of thrombophilia. Several studies have confirmed that the FVL (G1691A) mutation is a gene variant associated with an increased risk of thrombophilia in various ethnic populations. According to our findings, thrombophilia and FVL (G1691A) mutation were significantly associated $(P = 0.0045, OR = 8.83)$. This result aligns with a previous study conducted on populations in Tunisia and Lebanon, which demonstrated that the Factor V Leiden (G1691A) mutation was associated with thrombophilic defects and thrombosis (P < 0.001 for both populations), with calculated OR values of 6.3 and 5.1, respectively [28]. Furthermore, the Factor V Leiden (G1691A) mutation was significantly associated with thrombophilic defects (thrombosis) in the Tunisian population (P < 0.001) with a determined OR value of 6.1 [29]. A study conducted in an Iranian population similarly reported a positive correlation between FVL (G1691A) mutation and VTE [30]. Additionally, a study by Alfirevic et al., published in 2010, involving Croatian

patients diagnosed with VTE corroborated the association of the FVL (G1691A) variant with thromboembolic disease $(P = 0.004, OR = 6.41)$ [31]. Studies conducted in Lebanon [32], Kashmiri [8], and Turkey [18, 33] also showed similar outcomes (P<0.05). Our results contrasted with those reported by a study conducted in the Netherlands, which concluded that individuals possessing either homozygous or heterozygous Factor V Leiden (G1691A) mutation did not have a high risk of recurrent venous thrombosis [34].

5. CONCLUSION

The prevalence of thrombotic gene mutation (FVL) in Yemeni people with thrombophilia and the evaluation of the association between the FVL (G1691A) mutation and risk factors for thrombophilia (thrombosis) are reported for the first time in this study. Our study showed that the FVL (G1691A) variant is more prevalent in subjects with thrombophilia or VET than in healthy controls, which is in contrast to other published studies. This finding raises the possibility that this mutation increases susceptibility to thrombophilia (thrombosis). From the outcomes of this study, it can be concluded that the Factor V Leiden (G1691A) mutation was significantly associated with an increased risk of thrombophilia.

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