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Frequency of Factor V Leiden in Patients Presenting with Venous Thromboembolism

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Abstract

Objective: To determine the frequency of FVL mutation in patients of VTE presenting with or without predisposing environmental factors.
Methodology: It was a cross-sectional study conducted at Chughtai Institute of Pathology, from August 2022 to June 2023. The study included 80 adult patients aged 15 years and above and both genders, who were recently diagnosed with venous thromboembolism at any site in the body through radiological investigations (colored Doppler ultrasound) and put on anticoagulants. Data was collected from 80 diagnosed cases of VTE after informed consent over a period of 10 months. 5ml Blood sample in EDTA vial was analyzed for FVL using real time PCR after extracting DNA via kit method. The results were correlated with the clinical history and recurrence of the disease. Cases of VTE with or without any predisposing environmental factor and who were recently put on anticoagulant therapy were included in the study.
Results: FVL mutation was detected in 22.5% of the sample population affecting 18 individuals out of 80 patients with heterozygous mutation being more common. Recurrence was observed with FVL mutation suggesting significant association of recurrence with mutation (p value <0.05).
Conclusion: FVL mutation was found to be one of the common causes of VTE being identified in >20% of the population under study and was more likely associated with the recurrence of the disease in patients once their anticoagulants were put on hold.
Key Words: Inherited thrombophilia, Factor V Leiden (FVL), Venous thromboembolism (VTE), Recurrence, Anti-coagulant therapy.

Introduction

Venous thromboembolism is defined as "the development of clot in deep veins of the legs/arms (Deep Venous Thrombosis) and/or its separation from vessel wall, transportation in blood circulation carrying it to lungs (Pulmonary Embolism) producing characteristic sometimes fatal clinical features".[1] Venous thromboembolism due to its complications and association with genetic and acquired factors lead to an increased risk of mortality. It comes 3rd in the list of diseases causing death worldwide adding to the global disease burden.[2] The incidence of VTE varies across different regions of the world and is found to be relatively high in the western population (1-2/1000 persons in a year) as compared to eastern side of the globe.[3] Among multifactorial etiologies, the inherited tendency to develop VTE is mostly associated with a point mutation in Factor V gene (Termed as Factor V Leiden) leading to a confirmatory change that abolishes the cleavage site on activated Factor V rendering it resistant to be inactivated by activated Protein C, a natural anticoagulant.[4] Factor V Leiden results from a missense mutation commonly identified as c.1691G>A affecting 1 in 1000 individuals of general population in homozygous form with the incidence increasing to 1 in 100 patients presenting with venous thromboembolism.[5]

Meanwhile the heterozygous form of the factor V Leiden can manifest as VTE n 1 in 1000 carriers from minimum to a maximum of 3-8/1000 carriers annually.[5] Multiple thrombophilia provoking environmental factors in association with the already existing genetic factors can add to the annual increased risk of VTE as well as increased mortality of the disease.[6] These risk factors include pregnancy, hormones replacement, trauma and fracture with or without immobilization, long duration flights, obesity, smoking and cancer.[7]

Thrombophilia diagnosis not only requires detailed knowledge about the disease, but the comprehensive yet essential testing ordered by the treating physician. The facilities of lab analysis are substandard and lacking in our

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remote laboratories.[8] An early and accurate diagnosis can lead to early adaptation of treatment plans either temporary or permanent based on guidelines to avoid associated life threatening complications and recurrence of the thrombotic events.[9]

No significant study has been conducted in our population to determine the frequency of Factor V Leiden mutation (both heterozygous and homozygous) and its association with the presenting symptoms and recurrence of the disease. The aim of this study was to assess the disease burden in our society by determining the frequency of FVL mutation in patients presenting with venous thromboembolism and typing it as homozygous or heterozygous for early diagnosis and better management approach towards patients. We also aimed to determine the association of said mutations with clinical history and recurrences.

Methodology

It was a cross-sectional study conducted at Chughtai Institute of Pathology, from August 2022 to June 2023 after gaining approval from Institutional Ethical and Research committee. The study included 80 adult patients aged 15 years and above and both genders, who were recently diagnosed with venous thromboembolism at any site in the body through radiological investigations (colored Doppler ultrasound) and put on anticoagulants. Data was collected after informed consent based on a self-made questionnaire that included other co-existing environmental factor affecting the disease outcome such as smoking, obesity, hypertension, long standing hours, long haul flights, pregnancy, and hormone replacement therapy. 5ml of peripheral blood sample was taken in EDTA vial under aseptic conditions. DNA extracted via KIT method from white blood cells was amplified using real time polymerase chain reaction (RT-PCR). Gene specific primers and FVL c.1691G>A mutation specific fluorescence probe was used to detect mutant DNA/ FVL mutation against a background of wild-type genomic DNA.

Patients who were taking anticoagulants after being already diagnosed with acquired or inherited VTE were excluded from the study, only those patients who presented with VTE but no inherited or acquired cause was yet known were included in the study.

Descriptive statistics such as age were calculated as mean ±SD. Statistical analysis was performed using SPSS 23.0. Percentages of various risk factors in patients presenting with VTE was determined. Also, the frequencies of different clinical presentations were evaluated. Association between 2 groups (mutation positive and mutation negative) and recurrence was made using Chi-square test and p value <0.05 was set to be significant.

Results

Total 80 patients who presented to us with clinical picture of venous thromboembolism were included in the study out of which 42 were males and 38 were females. Mean age was 33.7±12.38 ranging from 10 years to 80 years (Table I). Factor V Leiden mutation was detected in 18 patients (22.5%), 2 patients had homozygous mutation of corresponding allele of Factor V gene while 16 patients were identified with heterozygous mutation of FVL allele. 62 patients (77.5%) had no FVL mutation detected.

Table I: Demographics and association of FVL mutated cases with recurrence.

Demographics	Total Population(n=80)
AGE	33.7±12.38
Gender	Total Population (n=80)
Mutation Negative (n=62)	
Male	45
Female	17
Mutation Positive (n=18)	
Male	12
Female	06
RECURRENCE	4/80 (5%)
P value = 0.005 (association of recurrence with mutation)	P<0.05*

**Chi Square test with p-value <0.05 being significant.*

Only 4 cases out of 80 patients complained of recurrence of disease and all of them were positive for FVL mutation. So, 4 out of 18 positive cases experienced recurrent thrombotic event amongst which only 1 patient had a predisposing factor. All of them were not on anti-coagulants at the time of 2nd thrombotic attack. All these patients having a recurrent thrombotic event were identified with heterozygous FVL mutation. Association between detection of mutation in 2 groups (1 group= mutation negative and 2 group= mutation positive) and recurrence was calculated by applying Chi-Square test and was found to be significant (p value < 0.05) Table I. No recurrence was found in other mutation negative patients.

In positive cases, 3 patients had 1st thrombotic event in the presence of a predisposing factor namely smoking, post intra-uterine fetal death and occupation associated long

duration standing hours while rest of the positive cases (15 out of 18) were unprovoked. Table II showcases the frequency of different risk factors in patients presenting with venous thromboembolism. Majority of patients presented with history of smoking, obesity, hypertension, and pregnancy while rest of the risk factors were less frequently found.

Table II: Frequencies of risk factors.

RISK FACTORS causing Provoked VTE	N (%)
Smoking	11 (13.7%)
Obesity	13 (16.2%)
Hypertension	15 (18.8%)
Long standing hours	5 (6.2%)
Long haul flights	4 (5%)
Pregnancy	15 (18.8%)
HRT	2 (2.5%)
TOTAL PROVOKED CASES	65 (81.2%)
TOTAL UNPROVOKED CASES	15 (18.8%)

Table III lists the clinical presentations of patients of VTE with majority suffering from Deep Venous Thrombosis accounting for 81% of total population studied. Rest is listed in the table 3 with respective percentages in patients of VTE. Out of 18 positive cases, 9 patients suffered from DVT (50%), 4 patients had cerebral vein thrombosis (22.2%), 3 patients experienced Pulmonary embolism (16.6%) and lastly 2 patients (11.1%) had visceral thrombosis.

Table III: Clinical presentation in VTE.

CLINICAL PRESENTATION (n=80)	Percentage
Deep Venous Thrombosis	81.3%
Cerebral Venous Thrombosis	6.3%
Pulmonary Embolism	8.8%
Visceral Thrombosis	3.8%

Discussion

Venous thromboembolism is a multi-causal disorder involving coagulation and fibrinolytic system of our blood and includes many genetically inherited mutations as well as environmental predisposing factors, both of which may co-exist adding to the mortality of the disease.[10] VTE sits at the 3rd place as one of the leading causes of death or if less severe, a physiologically crippled life making it a global burden whose prevalence however varies from country to country with majority of patients being identified in rather developed countries.[11]

Among all the genetically inherited mutations predisposing a patient to thrombosis, Factor V Leiden is most frequently associated with inherited thrombophilia affecting 8/1000 to 80/1000 individuals in its heterozygous and homozygous state respectively.[5,12]

To find the prevalence of this mutation in our area, we conducted a single center study that included 80 patients suffering from venous thromboembolism. The number of male individuals suffering from VTE was almost same as the female population (1.1:1). Gender is typically not associated with an increased or decreased risk of developing VTE though females may have an increased risk with growing age. The disease affected all ages ranging from a 15-year teen to a 78-year-old female. The risk of developing VTE increases with increasing age for both males and females with older female population more likely to suffer from the disease.[13]

Out of 80 patients tested in our study, 18 patients (22.5%) were identified with FVL mutation with heterozygous allele detected in 16 patients (88.8%) and homozygous allele in 2 patients (11.2%). 62 patients (77.5%) suffering from VTE were not identified to be having FVL mutation. Previous studies in Pakistan have reported different frequencies of FVL mutation in patients of VTE in different regions of the country varying from 3% in healthy females of Sindh in 2023[14], 5% in Northern Pakistan in 2021[15], 13% in a study done at AFIP in 2013[16] and 18% in another study done at AFIP in 2019.[17] Our study as being the latest one in this cause showed the greatest frequency of FVL mutation in patients of VTE yet reported in Pakistan. This could be due to a better understanding of inherited thrombophilia by treating physicians, leading to an increasing trend of thrombophilia testing done so that more such cases can come out and be appropriately treated.

Our study found 2 out of 18 positive cases to have homozygous mutation (11.2%) while rest of positive cases were identified with heterozygous mutation (88.8%). This exact distribution of type of mutation was found in another study done in Turkey by Yildiz et al.[18] Homozygous cases had no predisposing factors while 3 out of 16 heterozygous cases had 1 factor each predisposing them to VTE namely smoking, post IUD and long-standing hours. The risk of developing a thromboembolic event or recurrence gets increased in the presence of a predisposing factor.[19]

The recurrence rate of 2nd thrombotic event after 1st one was 5% (4 out of 80) and 22.2% (4 out of 18) in our study with all the cases having heterozygous mutation. Only 1

patient had a predisposing factor (smoking). The recurrence took place in the same site as the previous one at the time when the patients were put off the anti-coagulant therapy. Though heterozygous mutation in the absence of a predisposing factor poses decreased annual risk of developing VTE but in a case presentation by Mehmoud A et al.[20], a patient with heterozygous mutation developed multiple ocular thrombotic events both arterial and venous. Both these studies suggest high risk of recurrence with only heterozygous mutation raising the question about the short-term use of anti-coagulation therapy in such patients.

Our study also determined the association of recurrence in both groups (i.e Mutation Positive and Mutation negative Groups). Significant association of recurrence was found with mutation positive group. This means that patients with Factor V Leiden mutation have higher chances of developing recurrent VTE. BCSH guidelines suggest 3 months of anti-coagulant therapy for secondary VTE extending to 6 months for idiopathic VTE and life-long therapy with DOACs in case of recurrence. The decision of continuing anticoagulation therapy beyond 6 months is mainly based on Patient's preferences being well informed about the benefits and risks of treatment. Long term therapy after 1st thrombotic event may reduce the risk of recurrence by 80%, also as prophylactic therapy may be required in symptomatic patients.[21]

Coming towards the distribution of VTE within various sites of the body, 9 patients (50%) out of 18 positive cases had Deep Venous Thrombosis, 4 patients (22.2%) had cerebral vein thrombosis, 3 patients (16.6%) experienced pulmonary embolism and lastly 2 patients (11.1%) had visceral thrombosis. Another study by Federici et al. showed distribution pattern and frequency of VTE in different sites of the body. Out of 29 patients with homozygous FVL mutation, 62% patients presented with deep venous thrombosis (DVT) and 14% with pulmonary embolism (PE) and 24% with both DVT and PE.[22]

Conclusion

FVL mutation was found quite prevalent affecting 22.5% of the population presenting with VTE and with both heterozygous and homozygous mutations existing in our population. Recurrence was found to be associated with mutation detection. Carrying only heterozygous allele does not only put the person on risk of developing 1st unprovoked VTE but also increases the risk of recurrence. Therefore, screening and DNA testing for FVL mutation should be considered in all the cases of VTE followed by long term anticoagulation to prevent future complications.

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