FV Leiden, Prothrombin II, and MTHFR C677T Mutations in Children and Young Adults with Thromboembolic Diseases

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Abstract

Background: The main markers for estimation of the tendency for thromboembolic disorders are Factor V Leiden, Prothrombin gene (G20210A), and MTHFR (C677T) polymorphism. **Objectives:** The aim of the present research is to determine the frequency distribution of genetic mutations of (FV Leiden, Prothrombin II, and MTHFR) genes in patients with early-onset thromboembolic diseases. **Materials and Methods:** This is a retrospective study done on children and young adults of both sexes aged from 1 to 45 years with thromboembolic disease with no obvious reason who attended patients clinics in Al-Salam Teaching Hospital and were referred from private clinics for a period from June 1, 2019, to August 30, 2020. Detection of gene polymorphism was done in three steps started by isolation of DNA from the blood samples followed by *in vitro* polymerase chain reaction amplification then hybridization of amplification products at 45°C. **Results:** A total of 50 cases with thromboembolic diseases were enrolled in this study; 21 (42%) were male and 29 (58%) were female. Patients' mean age was (26.64 ± 8.68) years. Factor V Leiden mutation was the most frequent mutation (12%) followed by MTHFR (C677T) mutation 6%, and the least frequency was for prothrombin gene mutation G20210A (2%). **Conclusion:** The prevalence of mutations in gene encoding Factor V Leiden was higher than MTHFR C677T and Prothrombin 20210A polymorphisms in our locality, consequently, assay for mutation of Factor V Leiden must be included in the evaluation of patients with thromboembolic diseases with no obvious cause.

Keywords: Adults, children, Factor V Leiden, MTHFR (C677T), polymorphism, Prothrombin gene (G20210A), thromboembolic diseases

Introduction

Thromboembolic disease is a paramount contributor to mortality and morbidity worldwide.[1] The tendency to develop arterial or venous thrombosis by increasing hypercoagulability is defined as Thrombophilia.[2] Until age of 40, the most frequent form of thrombosis is venous thrombosis, after that the occurrence of arterial thrombosis increases and becomes more prominent.[3] Venous thromboembolism presents itself in form of either deep vein thrombosis or pulmonary.[4]

Both genetic and environmental factors cooperate in determining thromboembolism risk.[5] The inherited hypercoagulable states are linked with venous rather than arterial thrombosis and it is now documented that genetic basis is existent in the majority, if not all, patients with thromboembolism.[6]

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The most common Genetic variants that predispose to hypercoagulable state and thrombosis are Factor V Leiden G1691A (*FVL*), prothrombin G2021A (*PT20210A*), and methylenetethraydrofolate reductase C677T (*C677T MTHFR*).[7]

Factor V Leiden is a hereditary blood clotting disorder.[8] A single-point mutation in the exon 10 of the FV gene causes this mutation.[9] FVL is inherited by an autosomal dominant manner and it is the most common genetic risk factor for VTE.[10] It causes an insufficient anticoagulant

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response to activated protein C and leading to increase the risk of pulmonary embolism and deep vein thrombosis among patients with Venous thromboembolism.[11] Venous thrombosis risk in heterozygous carriers increases in 3–7 times and may reach 80 times in homozygous people.[12]

Another genetic risk factor is a point mutation of prothrombin gene which has been associated with a two-fold increased risk of venous thrombosis by increasing the plasma prothrombin levels higher than normal.[13]

A reduction in MTHFR enzyme activity and moderately-raised homocysteine level is mostly caused by C677T polymorphism of MTHFR gene.[14] This polymorphism influences the pathogenesis of thrombosis and correlates with venous and arterial thrombus development.[15]

The aim of the present study is to determine the frequency distribution of genetic mutations of (FV Leiden, Prothrombin II, and MTHFR) genes in patients with early onset thromboembolic diseases with no obvious causes.

Material and Methods

This is a retrospective study. Its sample includes children and young adults of both sexes aged from 1 to 45 years with attack of thromboembolic disease with no obvious reason and no family relationship who attended patients clinics in Al-Salam Teaching Hospital and were referred from private clinics for a period from June 1, 2019, to August 30, 2020.

Patients aged above 45 years, Presence of other diseases: malignancy, hypertension, diabetes mellitus, inflammatory bowel disease, women on oral contraceptives, and pregnant, were excluded in this study.

All suspected patients were physically examined by physicians and the diagnosis of thrombotic disorders was predicted on combination of patient history, clinical examination, and laboratory evaluation.

"Blood sample was drawn from the participants and collected in EDTA blood collection tubes, and kept at room temperature and treated within two hours of collection. Genomic DNA was isolated from whole blood using Genomic kit (FV-PTH-MTHFR Strip Assay from ViennaLab) based on polymerase chain reaction (PCR) technologies. Extraction starts with incubating 100 µL of the whole blood and Lysis Solution for 15 min, that is, followed by a binding step using the GenXTRACT Resin and another incubation. Taq DNA Polymerase was diluted to 0.2 U/µL by using the Dilution buffer. The final total PCR volume is 25 µL: 15 µL is made up of Amplification Mix and 5 µL of diluted Taq Pol and 5 µL is made of extracted DNA. Finally, the amplification products are hybridized to a test strip, which contains (wild and mutant-specific probes) immobilized as parallel lines. The assay covers three mutations: FV (G1691A), PTH (G20210A), and MTHFR (C677T)."[16]

Statistical analysis

The collected data were analyzed by using suitable software program SPSS version 20. Tables of frequencies and proportions were used to express the outcome measures.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 16 on May 27, 2019.

Result

A total of 50 patients with thromboembolic diseases were enrolled in this study; 21 (42%) males and 29 (58%) females. The mean age of patients was (26.64 ± 8.68) years. The age distribution of the patients were shown in Figure 1.

None of the participants had positive family history of thromboembolic diseases.

The most frequent clinical features of thromboembolic disease patients was deep vein thrombosis 15 (30%) followed by cerebrovascular accident 14 (28%) and pulmonary embolism 5 (10%) [Table 1].

Table 2 shows the frequency of gene mutations in patients with thromboembolic disorders, 6 (12%) patients had heterozygous FV Leiden mutation; 3 (6%) patients had C677T MTHFR mutation, and only 1 (2%) patient showed Prothrombin II mutation. Combined FV Leiden with C677T MTHFR mutations were found in 3 (6%) patients, while 4 (8%) patients presented with a combination between FV Leiden and prothrombin II. On the other hand, Prothrombin II G20210A mutation, presented in 1 (2%) patient combined with low protein S level [Figure 2]. No significant differences in these mutations were found between males and females.

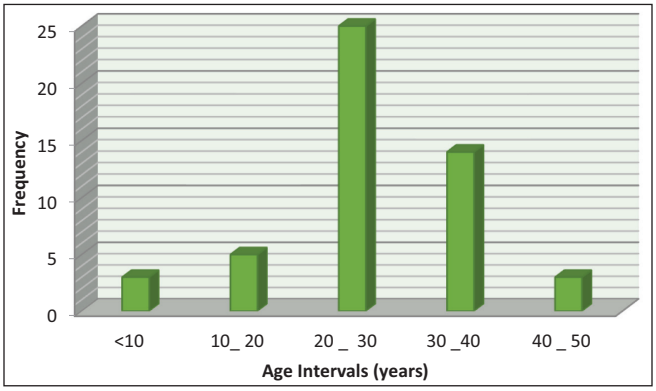

Figure 1: Age distribution of patients with thromboembolic disease

Discussion

Factor V, Factor II 20210A, and MTHFR (C677T), gene polymorphisms are genetic risk factors associated with hereditary thrombophilia; that is associated with a significant morbidity and mortality.[17] This study was conducted to estimate the frequency of FVL/FII G20210A/MTHFR C677T mutation in children and young patients with thromboembolic disorders with no obvious causes.

Table 1: The frequency of clinical features in patients with thromboembolic diseases

Diagnosis	No.	%
CVA	14	28
PE	5	10
PE and DVT	3	6
DVT	15	30
Mesenteric vein thrombosis	3	6
Portal vein thrombosis	3	6
Loss of vision	2	4
Repeated abortion	2	4
TIA	1	2
DVT and CVA	1	2
TIA and loss of vision	1	2
Total	50	100%

CVA: cerebrovascular attack, PE: pulmonary embolism, DVT: deep vein thrombosis, TIA: transient ischemic attack

Table 2: Frequency of gene mutations in patients with thromboembolic disorders

Cause of thrombosis	No.	%
AT III	3	6
FV Leiden	6	12
Autoimmune antibodies (ANA, APL)	10	20
FV Leiden + C677T MTHFR	3	6
FV Leiden + Prothrombin II	4	8
C677T MTHFR	3	6
Protein S deficiency	5	10
Prothrombin II	1	2
Prothrombin II + Protein S	1	2
Unknown	14	28
Total	50	100

AT III: Antithrombin III

In the present study, DVT was most frequent clinical presentation of the patients followed by CVA and PE; these findings are inconsistent with that obtained from another study done by Yokus *et al.*[18] who reported that CVA is the most frequent presentation of young patients with thromboembolic disorders followed by recurrent abortion and retinal vein thrombosis.

Regarding the mutant hematological variants, heterozygous FVL was the most frequent (12%) mutation in our patients, followed by C677T MTHFR (6%), and the lowest frequency was for Prothrombin II mutation (2%). These findings are similar with Elassal *et al.*[19] who find that FVL is the most frequent mutation followed by mutant C677T MTHFR gene, then Prothrombin II 20210A gene mutation; however our results come in contrary with other study done by Ozturk *et al*. which revealed that C677T MTHFR mutation is the most frequent defect followed by heterozygous FVL then Prothrombin II mutation.[20]

Combined FVL and Prothrombin II mutation was noticed in 8% of patients. This combination is important for the assessment and management of thrombophilic patients. Identification of these mutations helps in identifying high-risk patients and assessing the interaction between acquired and genetic risk factors.[21]

Other combination between FV Leiden and MTHFR genotyping was reported in 6% of our patients; which plays a synergistic role in thrombotic disorders leading to worse prognosis and even death in some patients as noticed by Bansal.[22]

In addition to that Prothrombin II mutation with protein S deficiency was identified in 2% of patients; however, people who carry two disorders appear to be at a greater risk for thrombotic diseases than those with a single mutation.[23]

Antithrombin (AT), and protein S are natural anticoagulant proteins which have a significant role in the regulator of thrombus formation and propagation. Inherited deficiencies of these proteins signify a strong risk

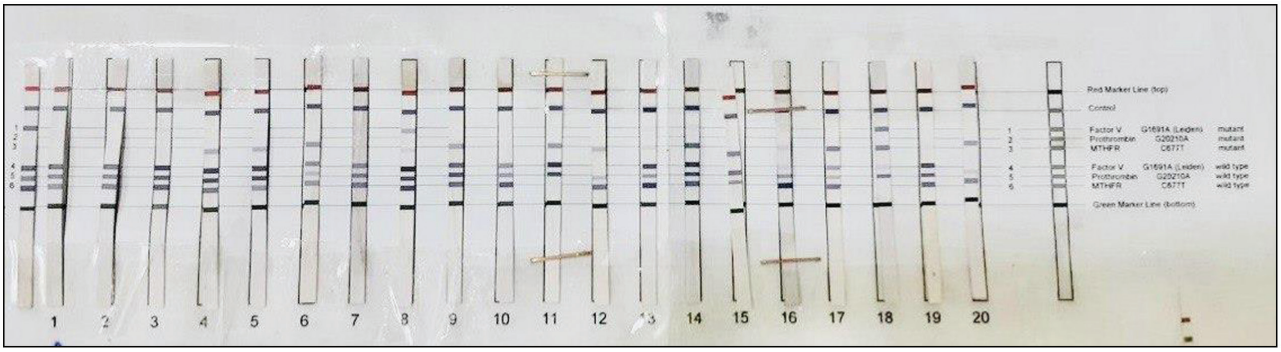

Figure 2: Polymerase chain reaction results of FV Leiden, Prothrombin II, MTHFR C677T, mutations in patients with thromboembolic diseases

factor for specific types of inherited thrombophilias.[23,24] In the present study; 10% of cases had Protein S deficiency and 6% of them had AT deficiency.

Autoimmune antibodies are diagnosed in 20% of the thromboembolic patients in our study. There is a tight link between The immune and coagulation systems, and it is seen that immune-mediated disorders are associated with an increased risk of venous thromboembolism in comparison to other medical causes of hospital admission.[25]

Nonsignificant differences were found between both genders in the frequencies of thrombophilic abnormalities as it is detected by Paschoˆa AF and Guillaumon AT.[26]

The findings of this research are contextualized in scope of certain limitations; it is just a retrospective descriptive study and the lack of control group in this design make difficult to formulate clear association between thromboembolic events and these mutation likewise the small sample size which need to be overwhelmed in future studies.

Conclusion

The prevalence of Factor V Leiden mutation was higher than MTHFR C677T and Prothrombin 20210A polymorphisms in our locality, consequently, assay for mutation in gene encoding for Factor V Leiden must be included in the evaluation of patients with thromboembolic diseases with no obvious cause.

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Conflicts of interest

There are no conflicts of interest.

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