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The Role of Factor V Leiden 1691G>A and Prothrombin Gene 20210G>A Mutations in Hypercoagulable State Associated with Venous Thromboembolism among Sudanese Patients

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Abstract

Background: Factor V Leiden (FVL) *1691G>A* and Prothrombin (PRT) *20210G>A* mutations are the most frequent hereditary cause of venous thrombosis in Caucasian and less frequency in African. The aim of this study was to detect the frequency of FVL *1691G>A* and PRT *20210G>A* mutations among Sudanese venous thromboembolism (VTE) patients.

Material and Methods: This was descriptive Cross sectional study in which a total of 176 Sudanese subjects were enrolled in the period between July 2015 and July 2016. Among them, 38 apparently healthy Sudanese individuals as controls and 138 patients (47 males and 91 females), age range 18-90 with documented VTE confirmed by Duplex Doppler ultrasound at Khartoum Teaching Hospital and Sudan Heart Institute were included.

Result: In this study, the mean age was 48 years and 67% of total VTE patients were over the age of 40 years. Increased age was noted in the VTE patients with over 34% over the age of 60 years. The controlled subjects included significantly younger individual with 92.1% under the age of 50 and 81.5% under the age of 40 years. The FVL *1691G>A* and PRT *20210G>A* mutations were totally absent among the studied population.

Conclusion: The FVL *1619G>A* and PRT *20210G>A* mutations were totally absent among Sudanese VTE patients.

Keywords: Factor V Leiden; Prothrombin gene; Venous thromboembolism; Sudan

Introduction

Venous thromboembolic disease (VTE) is a term includes deep vein thrombosis (DVT) and pulmonary embolism (PE), or a combination of both. DVT is a common vascular condition that arises from the formation of a blood clot within the deep veins of the circulatory system. PE occurs when a segment of that thrombosis detaches or separates from the vein wall, travels through the bloodstream, and lodges in the pulmonary artery [1]. Venous thrombosis is a serious health problem causing significant morbidity and mortality. It may be fatal by its complication of pulmonary embolism Fatality rate of venous thrombosis is estimated at 1% to 2% [2,3]. The pathogenesis of venous thrombosis is including acquired and genetic risks factors. Acquired risk factors including pregnancy, oral contraceptive use, estrogen therapy, obesity, malignancy, diabetic mellitus, immobility, trauma and post operation can precipitate thrombosis. Genetic risk factors include the deficiency of protein C, protein S, antithrombin and mutations of factor V Leiden and Prothrombin gene [4,5]. Both of these genetic abnormalities are commonly found in patients with VTE, with prevalence varying between 20% and 50% for factor V Leiden and between 5% and 19% for prothrombin gene mutation [6]. Previous research indicated that the presence of factor V mutation increases risk for venous thrombosis 7-fold in heterozygotes and 80-fold in homozygotes [7]. The incidence of venous thrombosis in homozygotes is almost 100% and 10% in heterozygotes.

Factor V Leiden Mutation (*1691G>A*): A single point mutation (G→A) at nucleotide position 1691 in the factor V gene results in a mutant form of factor V known as factor V Leiden [8]. This mutation results in a replacement of Arginine residue 506 with a Glutamine (R506Q) at one of the factor V cleavage sites for activated protein C (APC). Mutant factor V is resistant to inactivation by APC [4]. Several studies have demonstrated a relationship between the presence of APC resistance and an increased risk of venous thrombosis [9]. The FV Leiden allele is associated with a hypercoagulable state, which is reflected by increased levels of prothrombin activation fragments in plasma of individuals with inherited APC resistance [10]. The hypercoagulable state in carriers of FV Leiden mutation is explained by two mechanisms; In the first one APC cleavage site in FVa is lost, which impairs the normal degradation of FVa by APC [11]. The second observation is that FV Leiden is a poor APC cofactor in the degradation of FVIIIa because the cleavage at Arg506 is required for expression of APC cofactor activity of FV [12-14]. Molecular detection of FVL mutation could be achieved by different methodologies, the most common among them is PCR-RFLP [15].

The Factor II (Prothrombin) mutation *(20210G>A)* is the second most common genetic defect associated with inherited venous thrombosis. The genetic variation (Guanine to Adenine transition at position 20210) in the 3'-untranslated region of the prothrombin gene had been associated with elevated blood levels of prothrombin which

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leads to a state of hypercoagulability, and increased incidence of venous thrombosis [5]. Some individuals who have Factor V Leiden mutation may also have the Prothrombin *20210 G>A* mutation. Women with prothrombin *20210 G>A* are at a 16 times greater risk of developing VTE if they use estrogen based birth control pills. It is also advised that women with this mutation avoid hormone replacement therapy [16,17].

Aim

To determine the frequency of FVL *1691G>A* and PRT *20210G>A* mutations among Sudanese venous thromboembolism patients.

Materials and Methods

This was a descriptive, Cross sectional study, in which a total of 176 Sudanese subjects were enrolled in the period between July 2015 and July 2016. Among them, 138 patients (47 males and 91 females), age range 18-90 with clinical features of VTE confirmed by Duplex Doppler ultrasound at Khartoum Teaching Hospital and Sudan Heart Institute were included. Clinical data including age, sex, personal and family history for thromboembolic evidence, known risk factors for venous thromboembolism including surgery in the previous 3 months, child birth, pregnancy, malignancy and lengthy air travel (over 6 hours) were collected. The study included 38 apparently healthy Sudanese subjects as controls. Controls were free from history of venous thromboembolism, personal or family history of VTE, blood coagulation disorders, coagulation related medication and risk factors of VTE. The study was approved by the ethical committee of medical researches at Al Zaiem Al Azhari University. Informed consent was taken from participants before enrollment in this study.

Statistical analysis

All categorical variables were analyzed by descriptive statistics. Numerical variables were presented as Mean ± SD.

DNA extraction

Genomic DNA was extracted from peripheral blood samples collected from patients and control using Aidlab Genomic extraction Kit (China) according to the manufacture protocol.

PCR-RFLP for identification of FVL mutation

G to A substitution at nucleotide 1691 which is located in exon 10 of factor V gene was determined by PCR-RFLP method and published primer sequence described by Nahid et al. [18].

Briefly; PCR was performed in 4 µl premix tubes (iNtRON Biotechnology, Korea) in a total volume of 20 µl containing 4 µl genomic DNA, 10 µl DNAase free DW 10 pico mol of each primer (Forward 5'TCAGGCAGGAACAACACCAT3' and Reverse 5'GGTTACTTCAAGGACAAAATACCTGTAAAGCT3'). Reactions were carried out in a thermal cycler (Eppendorf tube). Thermocycling profile consisted of 5 min at 94°C followed by 35 cycles including 94°C for 30 s, 57°C for 30 s, and 72°C for 45 s. Samples were maintained for final extension at 72°C for further 10 min. PCR product was visualized by gel electrophoresis on 2% agarose gel (iNtRON Biotechnology, Korea) stained with Ethidium Bromide. Then 10 µl of each PCR product was digested with 10 units of Hind III in NEB Buffer for 18 hours at 37°C. Results of enzymatic digestion was separated by electrophoresis on 2% agarose gel (iNtRON Biotechnology, Korea) stained with Ethidium Bromide and the products were visualized in UV Transiluminator.

PCR-RFLP for identification of Prothrombin gene (*20210G>A*) mutation

G to A substitution at nucleotide 20210 which is located in exon 14 of factor II was examined by PCR-RFLP method. A region with 345 bp of exon 14 was amplified using following primers: Forward primer 5'TCTAGAAACAGTTGCCTGGC3' and Reverse primer 5'ATAGCACTGGGAGCATTGAAGC3' [18]. PCRs were performed in the same condition for factor V Leiden.

Results

The researcher describes characteristics of the samples and addresses the research questions. The data were collected for 138(65.9% females 34.1% males) with documented VTE, in this study. The mean age was 48, age range of 18-90, and 67% of total VTE patients were over the age of 40 years. Increased age was noted in the VTE patients with over 34% over the age of 60 years. The controlled subjects included significantly younger individual with 92.1% under the age of 50 and 81.5% under the age of 40 years. The variable frequencies of The past medical history for the overall participants included: without medical history 29%, post-operative disease (POD) 23% post natal 16.7%, hypertension (HTN) 5.8%, pregnancy 5.8%, thyroids disease 4.3%, diabetes mellitus (DM) 4.3%, malignancy 2.9%, liver disease 2.2%, cardiovascular disease (CVD) 2.2%, sickle cells disease (SCD) 1.4, cerebrovascular accident (CVA) 0.7%, respiratory disease 0.7% and systemic lupus erythrematosus (SLE) 0.7%.

PCR-RFLP for FVL (1691G>A) gene mutation

Amplification of a region of FV using the specified primers gave PCR products of 241 bp. In the normal gene there was no restriction site for Hind III in the 1691GG allele and the fragment of 241 bp was remained undigested, whereas 1691A allele was digested into two fragments of 209 bp and 32 bp. Both patterns were detected when the subjects were heterozygous (*1691GA*). When the DNA sample contains the FVL mutation in homozygous form (1691 AA genotype), only 209 bp fragment will be present in the gel (complete digestion).

The FVL *1691G>A* mutation was not detected in this study population. Represented samples are shown in (Figure 1).

PCR-RFLP for prothrombin (20210G>A) gene mutation

Polymerase chain reaction for *20210G>A* gene yields a product sized 345bp. In the normal gene there was no restriction site for Hind III in the 20210GG allele and fragment of 345 bp was remain undigested, whereas 20210AA allele was digested into two fragments of 322 and 23 bp. Both patterns were detected when subjects were heterozygous *(20210G>A)*. When the DNA sample contains the prothrombin mutation in homozygous form (20210 AA, genotype), only 322 bp fragment will be present in the gel (complete digestion).

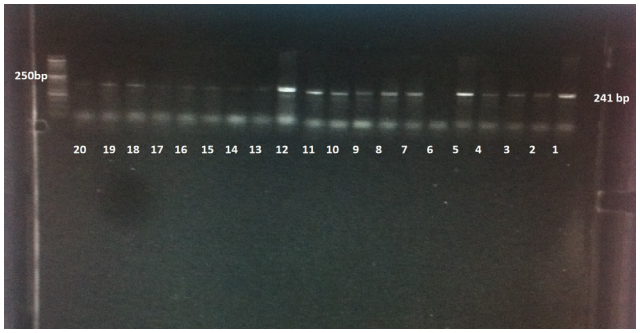

Figure 1: Shows Lane 21 is 50 bp DNA ladder. Lanes 1 up to 20: PCR products showing single band size 241 bp.

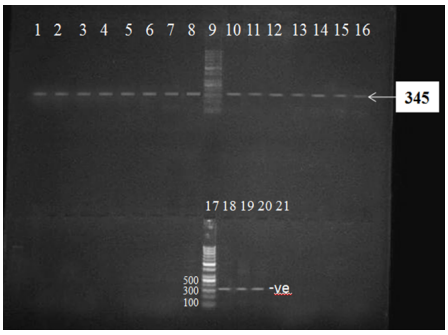

Figure 2: Shows representative 2% Agarose gel electrophoresis for PCR-RFLP of PRT mutation (20210 G>A). Lane 21: represents the negative control, lanes 1-20 except lanes 9, 17: represents the band size (345 bp) of PCR product, lanes 9 and 17: represents 100 bp DNA ladder.

The PRT *20210G>A* mutation was not detected in this study population.

Represented samples are shown in (Figure 2).

Discussion

The researchers determine the frequency of Prothrombin gene mutation and Factor V Leiden mutation in the Sudanese patients with venous thromboembolism.

Frequency of FVL varies from 0% to 15% according to ethnicity and geographic distribution worldwide [19]. FVL *1691G>A* mutation is higher in Caucasian than Non-Caucasian. Low frequencies were reported in African, Asian and South European populations (0%–3%). It is relatively high in North America (5%) and very high in Mediterranean populations (13.6% in Syria, 12.3% in Jordan, and 13.4% in Greece) [20]. PRT *20210G>A* is the second most common thrombophilic polymorphism in Caucasians, associated with the risk of thrombosis with an estimated population frequency of 2-3% in healthy people and 6.2% in VTE patients [21]. In comparison with previous studies conducted by Mathonnet et al. and Jun et al. [22,23] had similar results in the frequency of factor V Leiden and Prothrombin gene mutation. Mathonnet et al., Jun et al. reported FVL and PRT gene mutations to be totally absent in Moroccan an and Chinese VTE patients [22,23]. The findings of this study are in complete agreement with previous study reported by Mathonnet et al. and Jun et al. [22,23]. In addition to that, the method of the study was done by using PCR followed by *Hind* III digestion was also similar to Mathonnet et al. and Jun et al. Margaglione et al. found higher rates of FVL *1691G>A* and PRT *20210G>A* in VTE patients and controlled (12.1%), (7.1%) respectively [24,25]. The data result in this study differs with the finding of Margaglione et al. [24] and these discrepancies are most probably due to the small sample size, non-equal distribution of patient's age, ethnic affiliation and different geographical prevalence of these mutations. In Sudan, factor V Leiden gene mutation and prothrombin *20210G>A* gene mutation are not associated with VTE in Sudanese patients.

Conclusion

The FV Leiden *1691G>A* and PRT *20210G>A* mutations were totally absent in Sudanese VTE patients.

Conflict of Interest

The authors declared that they have no conflict of interest.

Author Contributions

Alfatih, Abdel Rahim and Prof Elwaleed designed the study; Alfatih performed the research; Ahmed Alhadi analysed the data, and Alfatih and Abdel Rahim wrote the manuscript. All authors approved the final manuscript.

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