ORIGINAL ARTICLE

Frequency of Factor V Leiden and Prothrombin G20210A Mutations in Sudanese Patients with Deep Vein Thrombosis in Khartoum Hospitals

AYMAN HUSSIEN ALFEEL[1], EZELDINE K. ABDALHABIB[2*], ABDULLAH A MOBARKI[1], HASSAN A HAMALI[1], IBRAHIM KHIDER IBRAHIM[3], SALAH ELDIN G ELZAKI[4]
[1]*Department of Medical Laboratory Technology, College of Applied Medical Sciences, Jazan University, KSA.*
[2]*Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, KSA.*
[3]*Department of Hematology, Faculty of Medical Laboratory Sciences, Al Neelain University, Khartoum, Sudan.*
[4]*Department of Epidemiology, Tropical Medicine Research Institute, Khartoum, Sudan.*
Correspondence to Dr. Ezeldine K. Abdalhabib, Email: email:ezeldine2008@yahoo.com

ABSTRACT

Background: Factor V Leiden (FVL) and prothrombin (PT) G20210A mutations play a crucial role in the development of deep vein thrombosis (DVT).
Aim: To determine the frequency of factor V Leiden and PT G20210A polymorphisms in Sudanese patients with DVT and the associated risks.
Methods: The current study was a hospital-based case-control survey, a total of 233 Sudanese subjects were enrolled within two groups: a patient group of 126 admitted with DVT and 107 age/ gender-matched healthy control group.
Results: Possible risk factors for DVT were found as a family history of thrombosis, hypertension, diabetes, smocking habitat, cardiac disease, and pregnancy. Heterozygous mutations of FVL was detected in one case (0.8%), and none of the studied subjects had PT G20210A mutation.
Conclusion: The current study suggests the presence of FVL and PT G20210A among patients with DVT. Further studies should be conducted to determine the prevalence of FVL and PTG 20210A mutations in a larger cohort.
Keywords: deep vein thrombosis, factor V Leiden, prothrombin G20210A

INTRODUCTION

Venous thromboembolism (VTE) is a common disorder of vascular system with many complications, including deep vein thrombosis (DVT) and pulmonary embolism (PE) (1–3). It is one of the leading causes of mortality and morbidity worldwide. It is estimated that 0.1% of adults develop VTE annually (2,3). VTE is a multifactorial disorder combining acquired conditions, circumstantial risk factors, and genetics (inherited) risk factors (2,4). Genetic risk factors represent 60% of VTE cases, which include Factor V Leiden (FVL), prothrombin G20210A (PT G20210A), protein C deficiency, protein S deficiency, and antithrombin deficiency. These genetics risk factors have been documented to be linked with an increased risk of VTE in many populations (5–10). FVL and PTG 20210A polymorphisms are the most common risk factors for VTE (11–13). FVL is found in 20–25% of patients with VTE and 50% of patients with familial thrombophilia (14,15), while the frequency of PT G20210A in the general population is 2.6%. The prevalence of FVL and PT G20210A mutations varies between ethnic groups and geographic distributions. The current study is aimed to determine the frequency of FVL and PT G20210A among Sudanese patients with lower extremity venous thrombosis.

MATERIALS AND METHODS

Patients selection: The current study was a case-control hospital-based study conducted from December 2016 to May 2017 in Khartoum hospitals. A total of 233 adults (aged from 40-70 years old) Sudanese subjects were enrolled within two groups: a patient group of 126 admitted with DVT confirmed by Doppler ultrasonography, and randomly selected 107 age- and gender-matched healthy individuals as a control group. The control group was selected based on no history of DVT. The study was approved by the Al Jazeera University Research Ethical Committee. Written informed consent was obtained from all participants.
Sample collection and DNA extraction: Venous blood was collected into ethylenediaminetetraacetic acid (EDTA) from the patients and the control groups. DNA was extracted and purified from blood samples by standard procedures using a master pure DNA purification kit G-DEXTMIIb Genomic DNA Extraction kit (Cat. No. 17241 Intron Biotechnology, Korea). Agarose gel electrophoresis was used to access the quality of the extracted DNA. Then DNA samples were routinely stored at -20°C.
DNA analysis: Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to analyze the genetic polymorphisms. PCR reactions were performed in a T100 thermal cycler (BioRad, USA) by use of 20 µl Maxime PCR PreMix Kit (i-Taq) (Cat. No. 25025 Intron Biotechnology, Korea). The primers that used in the amplification of PCR reactions were flank the region of mutation of FV and prothrombin gene. The sequence of the primers used for amplification of FVL and PT G20210A mutations were shown in table 1. Amplification products were digested at 37ºC, for 18 hours, with specific restriction enzymes: MnII and HindIII (New England Biolabs, USA) for FVL, Hind III (Fermentas, Lithuania) for PT G20210A. The digested products were subjected to electrophoresis using 2% agarose gel (Agarose LE, Intron Biotechnology, Korea).

DNA fragments were stained with 10 mg/ml of ethidium bromide (Promega, USA) The digested products were visualized by UV transellimantor and photographed. In the factor V gene, 241- base-pair (bp) segment was amplified using specific forward and reverse primers. The wild-type DNA 241, heterozygous yields two bands of 241, and 209 bp and homozygous mutation yield two bands of 209/32 bp. For detection of G20210A prothrombin gene mutation, a 345-bp genomic DNA fragment was amplified using specific primers; the wild-ty**p**e DNA yields a solitary 345-bp band, heterozygous yields two bands of 345 and 322 bp and homozygous mutation band of 322 bp and 23.

Statistical analysis: Statistical analysis was performed using GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA). Demographic data analysis was analyzed using the Chi-squared test or Fisher's Exact Test, as an appropriate unpaired t-test was used for other analysis. P values <0.05 were considered statistically significant. Mean±standard deviation (SD) were used for the presentation of all results unless otherwise stated.

RESULTS

In the current study, 126 patients and 107 matched healthy controls were enrolled. The participants included 126 DVT subjects with a mean age of 43.2 years old and 107 healthy control with a mean age of 40.1 years old. The male: female ratio among the patient group was found to be nearly equal, while among healthy individuals were (60.7%: 39.3%) (Table 2).

The baseline characteristic of the patient group and the control group are illustrated in table 3. In this study, several risk factors were observed with the patients, such as the family history of thrombosis, hypertension, diabetes, smoking, pregnancy, cancer, cardiac, and renal diseases. The family history of thrombosis was the highest risk factor, whereas cancer represented the lowest one, as indicated in table 3.

The prevalence of heterozygous factor V Leiden was 0.8% and homozygous was 0.0%. For prothrombin gene mutation, the prevalence of heterozygote and homozygote was 0% (table 4).

Table 1: Primer sequences for FVL and Prothrombin gene amplification.

Mutation	Forward Primer (5' ⟶ 3')	Reverse Primer (5' ⟶ 3')
FVL 1691G/A	TCA GGC AGG AAC AAC ACC AT	GGT TAC TTC AAG GAC AAA ATA CCT GTA AAG CT
FII 20210G/A	TCT AGA AAC AGT TGC CTG GC	ATA GCA CTG GGA GCA TTG AAG C

Table 2: Characteristics of age and gender among studied subjects.

	Patient group (n=124)	Control group (n107)
Mean Age (year old)	43.2 (40-70)	41.1 (40-70)
Male	64 (51.6%)	65 (60.7%)
Female	60 (48.4%)	42 (39.3%)

Table 3: Possible risk factors for DVT among studied subjects

Risk factor	DVT Patients	Controls	Relative Risk at 95 confidence level	P-value
Family history of thrombosis	30 (24.2 %)	0.00 (0.00%)	52.70	0.003
Hypertension	19 (15.3 %)	0.00 (0.00%)	33.69	0.014
Diabetes	19 (15.3 %)	0.00 (0.00%)	33.69	0.014
Renal disease	8 (6.5%))	0.00 (0.00%)	14.68	0.064
Smocking habitat	18 (14.5%)	4 (3.7%)	3.88	0.012
Cardiac disease	14 (11.3 %)	0.00 (0.00%)	25.06	0.025
Cancer	3 (2.4%)	0.00 (0.00%)	6.05	0.232
Pregnancy	6/60 females (10%)	0/42 females (0.00%)	20.96	0.037

Table 4: Factor V Leiden and prothrombin G20210A mutation in studied subjects:

	Patients	Controls	Odd Ratio	95 % CI:	P-value
FV leiden					
Homozygous GG	123 (99.2%)	107 (100%)	0.870	0.017- 44.2	0.558
Heterozygous GA	1 (0.8%)	00 (0.00%)	2.6	0.105- 64.8	
Homozygous AA	00 (0.00%)	00 (0.00%)	0.86	0.02 - 43.9	
Prothrombin G20210A					
Homozygous GG	124 (100%)	107 (100%)	0.86	0.02 to 43.9	0.942
Heterozygous GA	0(0.00%)	00 (0.00%)	0.86	0.02 to 43.9	
Homozygous AA	00 (0.00%)	00 (0.00%)	0.86	0.02 to 43.9	

DISCUSSION

Our study reports the prevalence of factor V Leiden and prothrombin gene mutations in patients with DVT in the Sudanese population. The prevalence of FV Leiden in heterozygous form was 0.8%. Venous thromboembolism (VTE), including DVT and PE complications, is a common disease, especially in the elderly[11]. Also, it is prevalent in African American populations compared to other communities. VTE is associated with poor quality of life and a reduced survival rate and has an economic burden on health care providers. It is estimated that 104-to 183 per 100,000 people per year develop VTE. In developing countries, DVT is the most common clinical manifestation of VTE and a leading cause of disability and death. The appropriate management of this disease requires rigorous knowledge of diagnostic and treatment modalities[16]. The mortality and morbidity of the disease are increasing every year, not only affecting African American populations but also increasing in other populations, including Asians and Caucasians. The incidence of VTE contrasts wide among various racial/ethnic cohorts; it seems global highest in

Blacks, is intermediate in Caucasians, and is lowest in Asians[17].

Furthermore, the prevalence rate of PT G 20210A and FVL mutations varies according to the ethnic and geographic distribution of the populations. Many recent studies have looked at the prevalence of mutations among the Chinese and Japanese populations. Our data demonstrate the presence of FVL in heterozygous form in 0.8% of patients with venous thrombosis. It is well-documented that the heterozygous form of FVL is associated with up to 7-fold increased risk of VE in healthy individuals and increased in the presence of other risk factors[15,18].

This study was shown that approximately 60% of the DVT patients were >40 years old; among them, about 20% of these patients were above 64 years old of age. These results were consistent with literature that stated the incidence of DVT increases with age for both secondary and idiopathic forms of DVT increasing nearly 90-fold in age between 15 and 80 years with a relative risk of 1.9 for each 10-year increase in age[19]. Reported risk factors for DVT vary widely; the significant acquired risk factors for venous thrombosis in this study were found as a history of venous thrombosis, hypertension, diabetes, smocking habitat, cardiac disease, and pregnancy. The understanding of the epidemiology and associated risk factors is equally essential to modify strategies in prevention, management, and treatment of VTE, predominantly in assessing patients with high-risk situations, and in defining the duration of anticoagulation required to decrease the occurrence of recurrent thrombosis and to avoid post-thrombotic syndrome[20].

Inherited risk factors of thrombophilic gene mutations are well known to have a contributory role in the occurrence of DVT. Factor V Leiden and prothrombin 20210G>A mutations which are the two most frequent DVT-associated polymorphisms26. These genetic polymorphisms leading to a hypercoagulable state and are thought to be responsible for the increased susceptibility to spontaneous or secondary venous thrombotic events at any age[21]. The prevalence rate of these mutations is very heterogeneous to the ethnic and geographic distribution of the populations28. In this regard, we designed this study to investigate the frequency and association of the two important polymorphisms with the manifestation of DVT in Sudanese patients.

The results of the present study showed that the frequency of heterozygous mutation of FVL (0.8%) in DVT patients with (0.00%) in the control group, while homozygous genotype for FVL was not detected. These results were agreed with the results of Awad-Elkareem *et al* (2017)[22] in which demonstrated a low-frequency rate of factor V Leiden mutation among Sudanese women with deep venous thrombosis during pregnancy and puerperium. Homozygous PT G20210A was found to be absent in studied subjects. Thus, the present study did not find any significant association of these genetic polymorphisms with thrombosis in Sudanese DVT patients. These results were in line with previous studies in DVT Sudanese patients, the same absent distribution was reported by Yousef et al. (2017)[23] found that: the FVL 1691G>A mutation was absent among the studied 138 VTE Sudanese patients and 48 control group. Another case-control study conducted in Sudan from July 2014 to July 2017 among 100 DVT patients and 92 control group, which aimed to investigate the prevalence of FVL (1691G>A) and prothrombin 20210G>A mutations in DVT patients, they were found none of the 192 subjects carried the Factor V Leiden or prothrombin 20210G>A mutations[24]. Furthermore, there was a study aimed to analyze the genetic and acquired risk factors for DVT of the lower extremities among Sudanese women which involved 75 DVT patients and 61 healthy controls both gene mutations were found to be absent from all 136 subjects. The current results were agreed with the reported data of a recent study on an African population in Senegal and Somalia in which both mutations did not detect in either patient with thrombosis or control group[25].

CONCLUSION

The current study suggests the presence of FVL and PT G20210A among patients with DVT. Further studies should be conducted to determine the prevalence of FVL and PTG 20210A mutations in a larger cohort.

Conflict of interest: The authors declare no conflict of interest.

Financial Support and Sponsorship: No fund resources

REFERENCES

1. Esmon C. Basic mechanisms and pathogenesis of venous thrombosis. Blood Rev. 2009;23(5):225–9.
2. Goldhaber S. Venous thromboembolism: epidemiology and magnitude of the problem. Best Pr Res Clin Haematol. 2012;25:235–42.
3. Khan S, Dickerman J. Hereditary thrombophilia. Thromb J. 2006;4(1):15.
4. Koster T, Rosendaal F, Brietetal EB. Protein C deficiency in a controlled series of unselected outpatients: an infrequent but clear risk factor for venous thrombosis (LeidenThrombophilia Study). Blood. 1995;85(10):2756–61.
5. Ten-Kate M, van der Meer J. Protein S deficiency: a clinical perspective. Haemophilia. 2008;14(6):1222–8.
6. Ho W, Hankey G, Quinlan D, Eikelboom J. Risk of recurrent venous thromboembolism in patients with common thrombophilia: a systematic review. Arch Intern Med. 2006;166(7):729–36.
7. Souto J, Almasy L, Borrell M, Blanco-Vaca F, Mateo J, Soria J, et al. Genetic susceptibility to thrombosis and its relationship to physiological risk factors: the GAIT study. Genetic Analysis of Idiopathic Thrombophilia. Am J Hum Genet. 2000;67(6):1425–9.
8. Larsen T, Sorensen H, Skytthe A, Johnsen S, Vaupel J, Christensen K. Major genetic susceptibility for venous thromboembolism in men: a study of Danish twins. Epidemiology. 2003;14(3):328–32.
9. Marchiori A, Mosena L, Prins M, Prandoni P. The risk of recurrent venous thromboembolism among heterozygous carriers of factor V Leiden or prothrombin G20210A mutation. A systematic review of prospective studies. Haematol 2007; 92 1107–1114. 2007;92(8):1107–14.
10. Lijfering W, Brouwer J, Veeger N, Bank I, Coppens M, Middeldorp S, et al. Selective testing for thrombophilia in patients with first venous thrombosis: results from a retrospective family cohort study on absolute thrombotic risk for currently known thrombophilic defects in 2479 relatives. Blood. 2009;113(21):5314–22.
11. Simone B, De Stefano V, Leoncini E, Zacho J, Martinelli I,

Emmerich J, et al. Risk of venous thromboembolism associated with single and combined effects of Factor V Leiden, Prothrombin 20210A and Methylenetethraydrofolate reductase C677T: a meta-analysis involving over 11,000 cases and 21,000 controls. Eur J Epidemiol. 2013;28(8):621–47.
12. Ridker P, Hennekens C, Lindpaintner K, Stampfer M, Eisenberg P, Miletich J. Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men. N Engl J Med 1995;332912–917. 1995;332(14):912–7.
13. Rosendaal F, Koster T, Vandenbroucke J, Reitsma P. High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). Blood. 1995;85(6):1504–8.
14. Segers K, Dahlbäck B, Nicolaes G. Coagulation factor V and thrombophilia: background and mechanisms. Thromb Haemost. 2007;98(3):530–42.
15. Franchini M. Utility of testing for factor V Leiden. Blood Transfus. 2012;10(3):257–9.
16. Klincheva M, Vilarova E, Angjusheva T, Milev I, Idoski E, Mitrev Z. Endothelial Nitric Oxide Synthase T-786C Mutation, Prothrombin Gene Mutation (G-20210-A) and Protein S Deficiency Could Lead to Myocardial Infarction in a Very Young Male Adult. Open Access Maced J Med Sci. 2016;15(4):142–5.
17. Martina Montagnana Emmanuel J. Favaloro Massimo Franchini Gian Cesare GuidiGiuseppe Lippi. The role of ethnicity, age and gender in venous thromboembolism. J Thromb Thrombolysis (2010) 29: 489-96.
18. Katcharin A, Napaporn A, Timaluck S, Pantep A. Prevalence of factor V Leiden (G1691A) and prothrombin gene mutation (G20210A) among different ethnic group in Thai hospitals. J Hematol Transfus Med. 2012; 22(2): 115-20.
A. Næss, S. C. Christiansen, P. Romundstad, S. C. Cannegieter, F. R. Rosendaal, and J. Hammerstrøm, “Incidence and mortality of venous thrombosis: a population-based study. Journal of Thrombosis and Haemostasis. 2007; 5(4) : 692–9.
19. Kreidy R. Influence of acquired and genetic risk factors on the prevention, management, and treatment of thromboembolic disease. Int J Vasc Med. 2014;http://dx.
20. De Stefano V, Rossi E, Paciaroni K, D'Orazio A, Cina G, Marchitelli E, et al. Different circumstances of the first venous thromboembolism among younger or older heterozygous carriers of the G20210A polymorphism in the prothrombin gene. Haematologica. 2003;88(1):61–6.
21. Awad-Elkareem A, Elzaki S, Khalid H, Abdallah M, Adam I. A low rate of factor V Leiden Mutation among Sudanese women with deep venous thrombosis during pregnancy and puerperium. J Obstet Gynaecol. 2017;37(7):963–4.
22. Yousif A, Muddathir A, Elamin E, Alhad A. The Role of Factor V Leiden 1691G>A and Prothrombin Gene 20210G>A Mutations in Hypercoagulable State Associated with Venous Thromboembolism among Sudanese Patients. J HematolThromb Dis. 2017;8:386–90.
23. Ibrahim N, Gameel F, Elgari M, Abdalla S. Evaluation of Genetic and Environmental Risk Factors for Deep Vein Thrombosis in Sudanese. J Clin Diagnostic Res. 2018;12(9):GC07-GC10.
24. Abdi A, Osman A. Prevalence of common hereditary risk factors for thrombophilia in Somalia and identification of a novel Gln544Arg mutation in coagulation factor V. J Thromb Thrombolysis. 2017;44(4):536–43.