FREQUENCY OF PROTHROMBIN GENE MUTATION IN VENOUS THROMBOEMBOLISM IN NORTHERN PAKISTAN

Zunera Sajjad, Saleem Ahmed Khan*, Hamid Saeed Malik, Chaudry Altaf, Nabeela Khan, Ayesha Khurshid

Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, *Army Medical College/ National University of Medical Sciences (NUMS) Rawalpindi Pakistan

ABSTRACT

***Objective*:** To determine the frequency of prothrombin gene mutation in venous thromboembolism in our population.

***Study Design*:** Cross sectional study.

Place and Duration of Study: Department of Haematology, Armed Forces Institute of Pathology Rawalpindi, from Jun 2015 to Dec 2016.

***Material and Methods*:** This cross sectional study was conducted in the Department of Haematology, Armed Forces Institute of Pathology Rawalpindi from Jun 2015 to Dec 2016. This study involved patients of both genders aged between 15-45 years who presented with one or more episodes of deep vein thrombosis or pulmonary embolism. Outcome variable was frequency of prothrombin gene mutation which was assessed by PCR of genomic DNA.

***Results*:** A total of 96 newly diagnosed patients of venous thromboembolism were analyzed. Out of these, 79 patients had deep vein thrombosis and 17 had pulmonary embolism. Prothrombin gene mutation was seen in 4 (4.2%) patients. The frequency of prothrombin gene mutation was significantly higher among patients with pulmonary embolism (p=0.002) and recurrent episodes (p=0.046).

***Conclusion*:** The frequency of prothrombin gene mutation was found to be 4.2% in the present study. It was significantly higher in patients with pulmonary embolism and recurrent thrombosis.

Keywords: Deep vein thrombosis, Pulmonary embolism, Prothrombin gene mutation

INTRODUCTION

Formation of a thrombus within a vessel and its detachment (embolus) from its site of origin is termed thromboembolism[1]. Venous thromboembolism (VTE) is an important clinical entity that is associated with significant morbidity and mortality[2]. According to western published data, annual incidence of first episode of venous thrombosis is 1.5 per 1000 person/year with a per person life time incidence of 5%[3]. Pulmonary embolism (PE) and deep venous thrombosis (DVT) are two common clinical presentations of the disease resulting from a complex interplay between genetic and environmental risk factors[4].

Genetic factors account for up to sixty percent of the risk of VTE, which comprises mainly the clinically evaluated Factor V Leiden 1691G>A and prothrombin 20210G>A[5]. The frequency of these mutations is up to 10% for Factor V Leiden and 3% for prothrombin G20210A in European populations[6]. Other factors include genetic deficiencies of proteins C, S, antithrombin, lupus anticoagulants, pregnancy, use of oral contraceptives, major surgery, cancer and inflammations[7]. The precursor of thrombin (Prothrombin) is a vitamin K-dependent protein which is synthesized in the liver and circulates with a half-life of three to five days[8]. Human prothrombin gene spans 21 kb on chromosome 11p11.2 and consists of 14 exons and 13 introns, which account for 90 percent of the sequence[9]. An extensive DNA sequencing of prothrombin gene has been carried out for patients with unexplained VTE and found a single missense mutation (guanine to adenine; G→A) at nucleotide position 20210, which is present in the 3′ untranslated region of the gene[10].

Correspondence: Dr Zunera Sajjad, Department of Haematology, AFIP Rawalpindi Pakistan *(Email: zunerashahbaz@gmail.com)*
Received: 09 Nov 2017; revised received: 24 Nov 2017; accepted: 29 Nov 2017

Patients with 20210G>A mutation frequently show plasma prothrombin levels three times higher than normal by increasing mRNA and protein expression for prothrombin. Moreover, increased prothrombin levels leads to an increase in a protein called thrombin-activatable fibrinolysis inhibitor (TAFI), which is an inhibitor of the fibrinolysis. Therefore, an increase in TAFI may disturb process of fibrinolysis allowing accumulation of clots leading to VTE[10]. Heparin, fondaparinux, dabigatran and warfarin are the commonly used anticoagulants. The aim of our study was to determine the frequency of prothrombin gene mutation in patients of venous thromboembolism and to study its association with different sites and episodes of thrombosis.

MATERIAL AND METHODS

This study was a cross-sectional analysis conducted at department of Haematology Armed Forces Institute of Pathology from July 2015 to October 2016. All male and female patients between 15 to 45 years who were diagnosed as Deep Vein Thrombosis or Pulmonary Embolism with single or recurrent episodes were included in the study. The diagnosis was confirmed on Doppler ultrasound and CTPA. Patients with any obvious cause of thrombosis like pregnancy, obesity, females on oral contraceptives and history of recent surgery were excluded. After informed and written consent complete physical examination, Doppler Ultrasound and CT Scan were performed. Complete blood counts, D-dimers and prothrombin gene mutation analysis by Polymerase Chain Reaction were also performed on blood samples.

Three 3ml of whole blood was collected in tube with EDTA anticoagulant. DNA of all patients was extracted from peripheral blood lymphocytes utilizing chelex resins. Prothrombin gene was amplified by a set of two primers. The DNA was amplified in a 20µl reaction mixture in an ependorff tube containing 20 pmol each of forward and reverse primers, 0.5 units of Taq polymerase (Fermentas life sciences, Lithuania), PCR master mix containing (30 mmol of each dNTP, 10 m mol tris HCl (pH 8.3), 50 mmol KCL, 2.0 mmol MgCl2, 10 mg/ml gelatin and 0.1-0.3 mg of genomic DNA. Thermal cycling comprised of initial denaturation at 94 C for 60 seconds, annealing at 65°C for 60 seconds and extension at 72°C for 90 seconds. These three steps consisted of 25 cycles. Final extension was carried out at 72°C for 3 minutes. The PCR amplified products, along with wild type, heterozygous and 100 bp ladder were electrophoresed on 6% polyacrylmide gel at 200 V for 25 minutes and later stained with silver nitrate for adequate visualization.

Data Analysis

Statistical package, SPSS version 24, was used for statistical analysis. Parameters for analysis included age, gender and gene mutation. Results were expressed as frequencies. Chi-square test was applied to calculate the *p*-values. A *p*-value less than 0.05 was considered statistically significant.

RESULTS

A total of 96 patients were diagnosed as having venous thromboembolism during the study period. Out of these 77 were diagnosed as deep venous thrombosis and 19 as pulmonary embolism. Successful molecular analysis was carried out in all patients. The male: female ratio was 4:1. The age range was 5 years to 45 years (median age of 33 years), Sixty seven patients presented with first episode while 29 patients presented with recurrent thrombosis. Prothrombin gene mutation was seen in 4 (4.2%) patients (table-I). Frequency of prothrombin gene mutation across site of thrombosis (table-II). The frequency of prothrombin gene mutation was significantly higher among patients with pulmonary embolism (17.6%) and in patients with recurrent venous thrombosis (table-III).

DISCUSSION

We present a comprehensive molecular data related to venous thromboembolism for patients investigated at our institute. Some of the significant observations are outlined below. In this study, mean age of the patients was 33.40 ±

7.69 years which was comparable to a study by Naqvi et al who reported mean age of 34.5 ± 11.5 years in Indian patients with DVT[11]. However, a relatively higher mean age of 42.53 ± 8.2 years has previously been reported by Saeed et al (2015) among such patients presenting at Armed Forces Institute of Pathology Rawalpindi[12]. A much higher mean age of 47 ± 8.29 years has been reported among such patients at Aga Khan University Hospital, Karachi[13]. We observed a male predominance with a male to female ratio of 4:1 which is in agreement with Shah et al (6:1)[14] and Saeed et al (3.1:1) in Pakistan and Khaladkar et al (2.9:1)[15] in India. Ballu et al (2013) observed much higher male predominance with a male to female ratio of 9.6:1 in Uganda[16]. Naqvi et al however reported a female predominance with a male to female ratio of 1:2.5[11].

In our study, 67 (69.8%) patients presented with first episode while 29 (30.2%) patients presented with recurrent thrombosis. Saeed et al also observed similar frequency of primary (60%) and recurrent thrombosis (40%) in patients presenting at Armed Forces Institute of Pathology Rawalpindi[12]. Prothrombin gene mutation was seen in 4 (4.2%) patients. Miles et al (2001) reported a frequency of 6.4% in American patients[17]. Attia et al (2009) observed a frequency of 3.33% in Egypt[18]. Dentali et al observed it to be 9.2% in Italy[19]. Yilmaza et al in 2014 reported the frequency of prothrombin gene mutation to be 11% in Turkish population[1] while Martinelli et al (2007) observed much higher frequency of 25% in Italian such patients[20]. The present study is first of its kind from Northern Pakistan and has found the frequency of prothrombin gene mutation to be 4.2% in thrombophilic patients reporting to our institute. This observed frequency of prothrombin gene mutation is comparable to other population with some differences which can be attributable to differences in population genetics. Our findings show significantly higher frequency of prothrombin gene mutation in patients with pulmonary embolism and recurrent

Table-I: Frequency of prothrombin gene mutation.

Prothrombin Gene Mutation	Frequency	Percentage (%)
Present	4	4.2
Absent	92	95.8
Total	96	100

Table-II: Frequency of prothrombin gene mutation across site of thrombosis.

Site	Prothrombin Gene Mutation		*p*-value
	Present (n=4)	Absent (n=92)	
Deep vein Thrombosis (n=79)	1	78	0.002
	1.3%	98.7%	
Pulmonary embolism (n=17)	3	14	
	17.6%	82.4%	
Total	4	92	
	4.2%	95.8%	

Table-III: Frequency of prothrombin gene mutation across number of episodes.

Episodes	Prothrombin Gene Mutation		*p*-value
	Present (n=4)	Absent (n=92)	
First (n=67)	1	66	0.046
	1.5%	98.5%	
Second (n=29)	3	26	
	10.3%	89.7%	
Total	4	92	
	4.2%	95.8%	

thrombosis which might suggest increased risk associated with the mutation. It is therefore advocated that screening of such patients for the presence of prothrombin gene mutation should be performed. This will identify patients at higher risk of recurrence and pulmonary embolism. Timely identification will help in anticipated management and reduction in morbidity and mortality rates associated with the condition.

CONCLUSION

We conclude that the frequency of prothrombin gene mutation is 4.2% in patients of venous thromboembolism in Northern Pakistan. It was significantly higher in patients with pulmonary embolism and recurrent venous thrombosis.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

REFERENCES

1. Yilmaz S, Gunaydin S. Inherited risk factors in low-risk venous thromboembolism in patients under 45 years. "Interact Cardiovasc Thorac Sur 2015; 20(1): 21-3.
2. Kupeli E, Verdi H, Şimşek A, Ataç BF, Eyüboğlu FO. Genetic mutations in Turkish population with pulmonary embolism and deep venous thrombosis. Clin Appl Thromb Hemost 2011; 17(6): E87-94.
3. Margaglione M, Grandone E. Population genetics of venous thromboembolism. A narrative review. Thromb Haemost 2011; 105(2): 221–31.
4. Raskob GE, Silverstein R, Bratzler DW, Heit JA, White RH. Surveillance for deep vein thrombosis and pulmonary embolism: Recommendations from a national workshop. Am J Prev Med 2010; 38(suppl-4): S502–9.
5. Zakai NA, McClure LA. Racial differences in venous thromboembolism. J Thromb Haemost 2011; 9(10): 1877–82.
6. Hosseeini S, Kalantari E, Dorgalaleh A, Rozei A, Jafari M, Naderi M, et al. Molecular Analysis Of Patients With Deep Venous Thrombosis. Blood 2013; 122(21): 4799.
7. Jadaon MM. Epidemiology of prothrombin G20210A mutation in the mediterranean region. Mediterr J Hematol Infect Dis 2011; 3(1): 467-92.
8. Miyawaki Y, Suzuki A, Fujita J, Maki A, Okuyama E, Murata M et al. Thrombosis from a prothrombin mutation conveying antithrombin resistance. N Engl J Med 2012; 366(25): 2390-6.
9. Miljić P, Heylen E, Willemse J, Djordjević V, Radojković D, Colović M, et al. Thrombin activatable fibrinolysis inhibitor (TAFI): A molecular link between coagulation and fibrinolysis Srp Arh Celok Lek 2010; 138(suppl): 74–8.
10. Alvarez SI, Ollero EB, LlinaresSanjuan FM, Martínez FL, Calvo Martín MT. A deep vein thrombosis caused by 20209 C>T mutation in homozygosis of the prothrombin gene in a Caucasian patient. Biochem Med (Zaqreb) 2014; 24(1): 159-66.
11. Naqvi SE, Haseen MA, Beg MH, Ali E, Khan T. Deep Vein Thrombosis: An Experience of 25 Years from North India. Indian J Vasc Endovasc Surg 2016; 3(1): 2-6.
12. Saeed A, Sumreen, Kashif MA. To determine the frequency of Factor V Leiden in cases of Deep Vein Thrombosis and Healthy controls. Pak J Med Sci 2015; 31(5): 1219-22.
13. Ali NS, Nawaz A, Junaid M, Kazi M, Akhtar S. Venous thromboembolism - Incidence of deep venous thrombosis and pulmonary embolism in patients with head and neck cancer: A tertiary care experience in Pakistan. Int Arch Otorhinolaryngol 2015; 19(3): 200-4.
14. Shah S, Bhalodia R, Patel K, Golwala PP. Prevalence of deep vein thrombosis with lower limb trauma patients. IntJ Biomed Adv Res 2015; 6(01): 9-15.
15. Khaladkar SM, Thakkar DK, Shinde K, Thakkar DK, Shrotri H, Kulkarni VM. Deep vein thrombosis of the lower limbs: A retrospective analysis of doppler ultrasound findings. J Med DYP Uni 2014; 7(5): 612-19.
16. Ballu S, Nyati M, Kajja I. Deep venous thrombosis in patients with acute traumatic spinal cord injury: prevalence and patterns in a major teaching hospital in Uganda. East Afr Orthop J 2013; 7(1): 36-43.
17. Miles JS, Miletich JP, Goldhaber SZ, Hennekens CH, Ridker PM. G20210A mutation in the prothrombin gene and the risk of recurrent venous thromboembolism. J Am Coll Cardiol 2001; 37(1): 215-8.
18. Attia FM, Mikhailidis DP, Reffat SA. Prothrombin Gene G20210A mutation in acute deep venous thrombosis patients with poor response to warfarin therapy. Open Cardiovasc Med J 2009; 3: 147-51.
19. Dentali F, Ageno W, Bozzato S, Malato A, Gianni M, Squizzato A, et al. Role of factor V Leiden or G20210A prothrombin mutation in patients with symptomatic pulmonary embolism and deep vein hrombosis: A meta-analysis of the literature. J Thromb Haemost 2012; 10(4): 732–7.
20. Martinelli I, Battaglioli T, Razzari C, Mannucci PM. Type and location of venous thromboembolism in patients with factor V Leiden or prothrombin G20210A and in those with no thrombophilia. J Thromb Haemost 2007; 5(1): 98-101.