ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 4 (1): 76-78, 2005
ISSN 1682-296X

Detection of the Factor V Leiden Mutation in Iranian Patients with Venous Thrombosis

[1]Rouzbeh Chegeni, [2,3]Bahram Kazemi, [4]Abbas Hajifathali, [1,6]Aliakbar Pourfathollah, [5]Ghasem Rastegar Lari, [6]Minoo Ahmadi, [6]Mohammadreza Tabatabaie and [7]Yadollah Mehrabi
[1]Department of Hematology and Blood Banking,
College of Medicine, Tarbiat Modarres University, Tehran, Iran
[2]Cellular and Molecular Biology Research Center, [3]Department of Parasitology, [4]Department of Hematology,
College of Medicine, Shaheed Beheshti University of Medical Science, Tehran, Iran
[5]Iranian Comprehensive Hemophilia Center, [6]Iranian Blood Transfusion Organization,
[7]Department of Health and Community Medicine,
Shaheed Beheshti University of Medical Sciences, Tehran, Iran

Abstract: Human coagulation factor V along with coagulation factor serves as a cofactor in thrombin formation by coagulation factor X. Generation of thrombin leads to fibrin and clot formation. In this study 45 patients were selected with venous thrombosis and evaluated first by the coagulation test for the presence of activated protein C (APC) resistance and then evaluated genetically by restriction enzyme analysis for the presence of factor V Leiden. 9(21%) patients had activated protein C resistance by the modified coagulation assay. When analyzed by restriction analysis, 7 (17%) had the factor V Leiden mutation. The present study shows that factor V Leiden has a lower frequency in the Iranian patients with venous thrombosis compared to the western societies.

Key words: Venous thrombosis, factor V Leiden, Activated Protein C resistance, Iran

INTRODUCTION

Human coagulation factor V acts as a cofactor in converting prothrombin to thrombin. In the coagulation process factor V is first converted to its active form, factor V_a in plasma, this is accomplished by factors X_a and/or α-thrombin[1]. Factor V is a single polypeptide chain molecule composed of 25 exons and 2224 amino acids. Upon activation by thrombin or factor X_a, factor V is cleaved into a dimmer composed of a heavy chain and a light chain. Factor V has six structural domains A_1, A_2, B, A_3, C_1 and C_2.

Once activated the B domain coded entirely by exon 13 is degraded. Activated factor V (FV_a) is a cofactor for factor X_a; together they form the prothrombinase complex, which activates prothrombin into thrombin leading to fibrin and clot formation. Factor V_a is quickly inactivated by activated protein C (APC) for maintenance of a balance in hemostasis[1]. The inactivation is a sequential process factor V_a is first cleaved at Arg 506 and subsequently at Arg 306 and Arg 679. Mutation in any one of these amino acids will predispose the affected individual to develop thrombosis a state called thrombophilia. APC resistance is present in 3 to 5% of asymptomatic Caucasians and is found in approximately 20% of unselected patients with venous thrombosis[2,3]. In at least 95% of cases resistance to APC is caused by a single point mutation of Arg 506 in the factor V gene[4]. A transition (G to A) at nucleotide 1691 in exon 10 results in the synthesis of a variant factor V molecule (Factor V Leiden) with the substitution Arg → Gln at amino acid position 506[5-7]. Patients with this genetic mutation may experience recurrent thrombotic episodes, throughout their lives. A heterozygous state for this mutation is associated with a 5 to 10 fold increase in the risk of thrombosis and a 50 to 100 fold increase for the homozygous state[8-10]. This mutation and APC resistance is extremely rare in Asian population[11]. The aim of this study was to evaluate the presence of Factor V Leiden in Iranian patients with venous thrombosis.

MATERIALS AND METHODS

Patients: APC resistance was measured on plasma samples from 45 patients by modified APC resistance assay after a diagnosis of deep vein thrombosis or pulmonary embolism was made. Deep vein thrombosis was diagnosed by ultrasound and venography and

Corresponding Author: Dr. Rouzbeh Chegeni, Department of Hematology and Blood Banking, College of Medicine, Tarbiat Modarres University, Tehran, Iran E mail: chegenr@yahoo.com

pulmonary embolism by ventilation-perfusion lung scanning. These patients were also evaluated genetically by restriction enzyme analysis for the presence of factor V Leiden mutation. Sample number was obtained by the statistical formula for estimated sample number.

Modified APC Resistance Assay: APC resistance in the presence of factor V-depleted plasma was assessed using the Coatest APC resistance-C kit and factor V-depleted plasma (Chromogenix)[12]. Plasma was prediluted 1 in 5 with factor V-depleted plasma and APC sensitivity ratios were determined as in standard assay. Modified APC sensitivity was less than 120 sec in patients and greater than 120 sec in normal individuals. Patients suspected of having the mutation by this coagulation test are analyzed genetically for the presence of factor V Leiden mutation.

Factor V exon 10 amplification: Genomic DNA was extracted from whole blood in each patient and a 380 bp DNA fragment, containing exon 10 of the factor V gene was amplified from genomic DNA by PCR. The amplification primers were designed corresponding to intronic sequences (forward primer: 5′-TTAGCCAGGCCTAACA-3′ and reverse primer: 5′-AATTGGTTCCAGCGAAAGCT-3′) of the exon 10 sequence. Each amplification reaction (50 μL) contained 0.5 μg of genomic DNA, 250 μmol L^{-1} of each deoxynucleotide triphosphate, 20 pmol of each amplification primer and 1.25 U of taq DNA polymerase (Cinnagen, Tehran) in 10 mMTris - HCl, 50 mM KCl, pH 8.3 and 1.5 mM $MgCl_2$. The amplification was performed with an Initial denaturation for 5 min at 94°C, followed by 30 cycles including denaturation at 94°C for 30 sec, annealing at 56°C for 40 sec and extension at 72 for 40 sec with a final extension for 5 min at 74°C[13]. At the end, 10 μL of the reaction mixture was analyzed by electrophoresis on a 2% agarose gel.

Restriction enzyme analysis: Restriction analysis for the detection of factor V Leiden mutation was performed as previously described[14] and the DNA product was digested with MnlI and analyzed by electrophoresis on 12% polyacrylamide gel.

RESULTS

Determination of APC resistance: Forty five patients diagnosed with venous thrombosis were chosen half male and half female. Seventeen had acquired thrombophilic risk factors such as using contraceptive pills, pregnancy, smoking and trauma. Their median age was 35 years. Eight patients had experienced venous thrombosis in the past. Their APC resistance time was between 60 to 120 sec. Nine patients (21%) had APC resistance, i.e. their APC resistance time was below 120 sec.

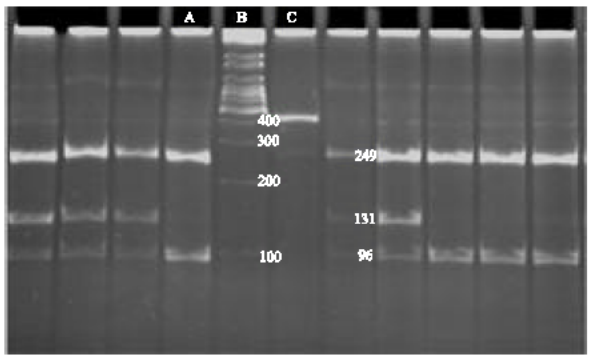

Fig. 1: Restriction analysis of Iranian factor V Leiden
Lanes 1-3: Heterozygous patients
Lane 4: Normal control (the 31 base pair fragment is not seen)
Lane 5: 100 bp DNA ladder marker
Lane 6: Uncut PCR product
Lanes 7-11: Heterozygous patients

Restriction enzyme analysis: The patients were analyzed by restriction enzyme analysis. Exon 10 and flanking intronic sequences were amplified producing a 380 base pair fragment. There are two cutting sites for restriction enzyme MnlI on exon 10. In factor V Leiden mutation one of these sites is destroyed. Individuals homozygous for the mutation will yield two fragments after digestion, a 249 and a 131 base pair fragment. Normal individuals will yield 3 fragments; 249, 95 and 31 base pairs in length - due to small size the 31 base pair fragment is not seen on the gel (Fig.1). Heterozygotes will have 4 fragments; 249, 131, 95 and 31 base pairs in length. Seven patients (17%) were heterozygous carriers of factor V Leiden by restriction analysis and homozygosity was not found.

DISCUSSION

In this study for the first time we have analyzed the presence of factor V Leiden in Iranian patients with venous thrombosis. Factor V Leiden which is a genetic mutant of coagulation factor V confers a thrombophilic state, there are other genetic risk factor as well predisposing an individual to develop venous thrombosis. Protein S, protein C and antithrombin deficiency are examples of important genetic risk factors. Acquired risk factor such as obesity, cancer, contraceptive pills, pregnancy and smoking also play a role in the development of venous thrombosis.

It is easy to see that venous thrombosis is a complex multifactorial disease. Several genetic and acquired risk factors must come together and through the interplay of these factors venous thrombosis develops.

Venous thrombosis annually affects 1 in 10000 persons younger than 40 years and 1 in 1000 persons older than 75 years of age causing significant morbidity and mortality[15]. APC resistance is now recognized as the most important cause of venous thrombosis present in up to 60% of patients with venous thromboembolism[15]. Differences in geographic distribution of APC resistance account for the wide frequency spectrum of APC resistance. In western societies APC resistance by factor V Leiden is more common than any other cause of venous thrombosis; therefore our aim was to see if factor V Leiden has the same frequency as in western societies. Zeinali *et al.*[16] studied the occurrence of factor V Leiden in an Iranian normal population and they reported a 5.5% frequency. The present report shows the frequency to be 17% in patients with venous thrombosis. When we analyzed APC resistance by the coagulation test, 21% of the patients showed resistance. This difference is probably due to some factors such as pregnancy, contraceptive pills and hormonal replacement therapy that can also cause APC resistance. It should be reminded that the main cause is factor V Leiden. Very lower frequencies have been observed in other parts of the world. In Asia Minor only 0.6%, the mutation was not found in Southeast Asia and Africa[16].

The present findings show that this mutation seems to follow a gradient pattern of distribution being highest in Europe specially Scandinavia and lowest in East Asia. Given our geographical location it is normal to find factor V leiden frequency in between the two poles of maximum and minimum frequency. Considering our sample size we feel larger sample numbers could give a more precise estimate of factor V Leiden mutation in the Iranian population with thrombosis.

REFERENCES

1. Chan, W.P., C.K. Lee, Y.L. Kwong, C.K. Lam and Raymond Liang, 1998. A novel mutation of Arg306 of factor V Gene in Hong Kong Chinese. Blood, 91: 1135-1139.
2. Koster, T., F. Rosendaal, H. de Ronde, E. Briet, J. Vandenbrouke and R. Bertina, 1993. Venous thrombosis due to poor anticoagulant response toactivated protein C: Leiden Thrombophilia study. Lancet, 342: 1503.
3. Svensson, P. and B. Dahlback, 1994. Resistance to activated proteion C as a basis for venous thrombosis. New Engl. J. Med., 330: 517.
4. Zoller, B., P. Svensson, X. He and B. Dahlback, 1994. Identification of thesame factor V gene mutation in 47 out of 50 thrombosis-prone families with inherited resistance to activated protein C. J. Clin. Invest., 94: 2521.
5. Bertina, R., B. Koeleman, T. Koster, F. Rosendaal, R. Dirven, H deRonde, P. van der Velden and P. Reitsma, 1994. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature, 369: 64.
6. Voorberg, J., J. Roelse, R. Koopman, H. Buller, F. Berends, J. Ten Cate, K. Mertens and J. Van-Mourik, 1994. Association of idiopathic venous thromboembolism with single point-mutation at Arg506 of factor V. Lancet, 343: 1535.
7. Greengard, J., X. Sun, X. Xu, J. Fernandez, J. Griffin and B. Evatt, 1994. Activated protein C resistance caused by Arg506Gln mutation in factor Va. Lancet, 343: 1361.
8. Bertina, R.M., B.P.C. Koeleman, T. Koster, F.R. Rosendaal, R.J. Dirven, H. de Ronde, P.A. van der Velden and P.H. Reitsma, 1994. Mutation in blood coagulation factor associated with resistance to activated protein C. Nature, 369: 64.
9. Svensson, P.J. and B. Dahlback, 1994. Resistance to activated protein C as a basis for venous thrombosis. New Engl. J. Med., 330: 517.
10. Dahlback, B., 1994. Physiological anticoagulation. Inherited resistance to activated protein C and venous thromboembolism. J. Clin. Invest., 94: 923.
11. Chan, L.C., C. Bourke, C.K. Lam, H.W. Liu, S. Brookes, V. Jenkins and J. Pasi, 1996. Lack of activated protein C resistance in healthy Hong Kong Chinese blood donors-Correlation with absence of Arg506-Gln mutationof factor V gene (letter). Thromb Haemost, 75: 522.
12. Jorquera, J., J. Montoro, M. Fernandez, J.A. Aznar and J. Aznar, 1994. Modified test for activated protein C resistance. Lancet, 344: 1162.
13. McPherson, M.J. and S.G. Moller, 2000. PCR. The Basics from Background to Bench. Bios Scientific Publishers. Chap. 2; Understanding PCR., pp: 9-21.
14. Beauchamp, N., M. Daly, K. Hampton, P. Cooper, F. Preston and I. Peake, 1994. High prevalence of a mutation in the factor V gene within the UK population: Relationship to activated protein C resistance and familial thrombosis. Br. J. Haematol., 88: 219.
15. Nicholaes, G.A.F. and B. Dahlback, 2003. Activated Protein C resistance (FV Leiden) and thrombosis: Factor V mutations causing hypercoagulable states. Hematol. Oncol. Clin. N. Am., 17: 37.
16. Rees, D.C., M. Cox and J.B. Clegg, 1995. World distribution of factor V Leiden. Lancet, 346: 1133.