INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

Short Communication

A CASE SERIES PROSPECTIVE STUDY OF FACTOR V LEIDEN MUTATION IN WEST BENGAL, INDIA

De Auley, Haldar Ajanta & De Madhusnata
Department of Genetics, Ramakrishna Mission Seva Pratishthan,Vivekananda Institute of Medical Sciences, 99 Sarat Bose Road, Kolkata-700026.

ABSTRACT
Venous thrombosis occurs due to alteration in either the vessel wall or flow of blood or in the coagulability of blood. A specific point mutation, called factor V Leiden mutation, responsible for abnormalities of coagulation cascade, increases the risk of venous thrombosis. Presence of factor V Leiden has been reported from the different parts of India, except from eastern India. A case series prospective study of thrombosis cases from West Bengal, India has been included. Restriction fragment length polymorphism of factor V gene was carried out in 12 duly screened & clinically diagnosed cases. Only 2/12 (16.6%) cases having repeated abortion with Deep Vein Thrombosis (DVT), had showed factor V leiden mutation. This study indicates the presence of factor V leiden mutation in West Bengal population among repeated abortion cases.

KEYWORDS: Deep Vein Thrombosis, Factor V Leiden, West Bengal.

INTRODUCTION

Venous thrombosis is a multifactorial disorder. Thrombosis occurs due to alteration in either the vessel wall or flow of blood or in the coagulability of blood. It is associated with a number of acquired and inherited factors. Deficiencies in the natural anticoagulants like protein c, protein s and antithrombin III caused thrombophilia. Activated protein C resistance is known to be a common and strong risk factor for thrombosis. (Dahlback B 1993, Koster T 1993, Griffin JH 1993) Certain genetic variants associated with abnormal haemostasis substantially increase the risk of venous thromboembolism in carrier because of defined biochemical alterations caused by the polymorphic factor V gene. A single point mutation occurred in the Exon 10 of factor V gene containing G →A substitution at nucleotide 1691 causing replacemen of arginine at 506 by glutamine Arg 506 is one of the three critical sites at which protein c cleaves and inactivate factor Va. As a result of this single point mutation called factor V leiden mutation, Gln506 from which is resistant to the activated protein c (Bertina RM 1994, Voorberg J 1994, Greengard JS 1994). This uncontroll coagulation cascade leads excessive thrombin generation that helps thrombosis. Although these mutation is predominantly found in Caucasian population (Bertina RM 1994, Rees DC 1995, Beauchamp NJ 1994, Svensson PJ 1994). This is very low in Asian population (Chan LC 1996). There is no such published report of genotype frequencies of factor V Leiden in these areas. The populations of these areas are untouched. The aim of this study is to investigate the frequency of factor five leiden mutation among eastern population.

MATERIALS AND METHODS

All the patients were obtained from Hematology Department after duly screened. Cases throughout the different part West Bengal were referred to the Hematology Department, Ramakrishna Mission Seva Pratisthan. These clinically diagnosed cases were mostly having one or more than one thrombotic risk factor like protein C, protein S, antithrombin III deficiency. Activated protein C resistance (Factor V Leiden) coagulation test reveals the normalized ratios below the decision limit. The level of these thrombotic risk factors were collected from their documented recent investigations. That may increase the chances of the presences of Factor V leiden mutation. The study was carried out in 12 patients having some problems like non hodgkin's lymphoma, repeated abortion, pulmonary embolism, myocardial infarction related with Deep vein thrombosis. All patients gave their written consent prior to the participation, and the study procedures followed were in accordance with the Institutional Ethical committee's guidelines. Patients with history suggestive of pancreatic disease or of ingestion of oral contraceptive drugs, or ultrasonographic evidence of liver tumor were excluded.

Collection of Blood

Whole blood samples of 12 patients were collected via Vacutainer blood collection tubes containing EDTA and stored frozen in microfuge tubes at –20°C.

Isolation of DNA

DNA was extracted from whole blood by salting out method (Miller S.A. 1988).

PCR-RFLP method to detect the genotype

The PCR amplification was carried out to identify the presence of G→A at nucleotide 1691 responsible for the factor V Leiden mutation as described by *Huber et al*. (Hurber S 2000) PCR product (241bp) was digested with HindIII restriction enzyme.

Agarose gel electrophoresis to detect the genotype

The mutation 241 G→A creates a HindIII recognition sequence, and the product was digested into 209bp and 32bp fragments. The products of restriction digestion were separated on 3% agarose gel and visualized by ethidium bromide staining.

TABLE 1: Percentage of Thrombotic markers present in DVT cases

Type of disease	Protein C deficiency n (%)	Protein S deficiency n (%)	Antithrombin III deficiency n(%)	Factor V leiden coagulation test n (%)	Factor V leiden mutation n (%)
DVT (n=8)	3(37.5%)	5(62.5%)	0	4(50%)	0
DVT with Acute Myocardial Infarction (n=2)	1(50%)	0	0	2(100%)	0
Repeated abortion due to Deep vein thrombosis (n=2)	1(50%)	1(50%)	0	1(50%)	2(16%)
Total cases (n=12)	5(41.66%)	6(50%)	0	7(58.33%)	2(16%)

TABLE 2. Different thrombotic markers of DVT cases

Type of disease	No. of cases	Age	Sex		Protein C value (Mean±S.E.)	Protein S value (Mean±S.E.)	Antithrombin III value (Mean ± S.E.)	Factor V leiden coagulation test (Mean±S.
			M	F				
Deep vein thrombosis (DVT)	8	34.25±4.58	5	3	93.81±9.23	80.16±7.88	117.68±6.22	1.25±6.87
DVT with Acute Myocardial Infarction	2	28±5.01	2	0	116.5±13.4	97±3.41	122.5±7.01	0.59±0.15
Repeated abortion due to DVT	2	34.5±2.50	0	2	87±7.31	85.1±7.14	100.7±5.73	0.88±8.51
Total	12	33.25±3.15	7	5	99.60±9.93	87.42±6.14	113.62±6.32	0.90±5.17

RESULT

This prospective case series study revealed that the protein c deficiency was present in 41.66%, protein s deficiency in 50% and antithrombin III in 0% of total studied cases. In the plasma based activated protein c resistance test, the reduced ratio (i.e. the ratio of activated partial thromboplastin time in the presence and absence of activated protein c) is found to be in 33.33% of the total population (Table. 1). Mean protein c level is 99.60±9.93, protein s is 87.42±6.14, antithrombinIII is 113.62±6.32. Factor V Leiden mutation was found in two (16%) cases of the tested twelve (Table. 2).

DISCUSSION

Factor five leiden mutation is common in western population, It is about 2-7% in Europian population while it is absent in Japanese population. (9,10) The risk of venous thrombosis increases approximately 5-10 fold for heterozygotes and 80 fold for homozygotes.(Bertina,1997) Factor five leiden mutation shows an uneven prevalence in different population of India. The study of Saxena et al showed 30.2% factor V leiden mutation in the deep vein thrombosis cases in North India (Saxena R.1999). In the similar type of patient group, Ghosh et al detected 3% factor V mutation in Western India (Ghosh K 2001). In Maharashtra this prevalence rate is about 2.4%-10.5% (Pawar A.R. 2001). Prevalence of factor V leiden mutation in south Indian population is approximately 15% for the case of deep vein thrombosis and 3% in the case of portal vein thrombosis (Koshy A. 2006).

The present case series prospective study on thrombosis patients of eastern India, presences of Factor V Leiden mutation has been reported. During analysis of the result it is found that all the cases were having one common problem that is deep vein thrombosis. But additionally they were having some more complication like two cases having repeated abortion, two cases were having acute myocardial infarction, one was having non hodgkin's lymphoma and one recurrent thrombo embolism. Rest six cases were having general deep vein thrombosis at the lower limb. Factor V Leiden was only found in the both repeated abortion cases. There is a chance of prevalence of Factor V Leiden mutation among the repeated abortion cases in West Bengal but further larger studies are required to stronger these findings.

REFERENCES

Dahlback, B., Carlsson, M., Svensson, P.J. (1993) Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: Prediction of a cofactor to activated protein C. *Proc Natl Acad Sci USA*, 90:1004.

Koster, T., Rosendaal, F.R., de Ronde, H., Briet, E., Vandenbroucke, J. P., Bertina, R.M. (1993) Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden thrombophilia study. *Lancet*, 342:1503.

Griffin, J.H., Evatt, B., Wideman, C., Fernandez, J.A. (1993) Anticoagulant protein C pathway defective in majority of thrombophilic patients. *Blood*, 82: 89.

Voorberg, J,, Roelse, J., Koopman, R., Buller, H., Berends, F., ten Cate, J.W., Mertens, K., Van Mourik, J.A. (1994) Association of idiopathic venous thromboembolism

with single point mutation at Arg506 of factor V. *Lancet*, 343:1535.

Greengard, J.S., Sun, X., Xu X., Fernandez, J.A., Griffin, J.H., Evatt, B. (1994) Activated protein C resistance caused by Arg506 Gln mutation in factor Va. *Lancet*, 343:1361.

Bertina, R.M., Koeleman, B.P.C., Koster, T., Rosendaal, F.R., Dirven, R.J., de Ronde, H., van der Velden, P.A., Reitsma, P.H. (1994) Mutation in blood coagulation factor associated with resistance to activated protein C. *Nature*, 369:64.

Rees, D.C., Cox, M., Clegg, J.B. (1995) World distribution of factor V Leiden. *Lancet*, 346:1133

Beauchamp, N.J., Daly, M.E., Hampto, K.K., Cooper, P.C., Preston, E., Peake, I.R. (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.

Svensson, P.J., Dahlback, B.(1994) Resistance to activated protein C as a basis for venous thrombosis. *N Engl J Med*., 330:517.

Chan, L.C., Bourke, C., Lam, C.K., Liu, H.W., Brookes, S., Jenkins, V., Pasi, J. (1996) Lack of activated protein C resistance in healthy Hong Kong Chinese blood donors—Correlation with absence of Arg506-Gln mutation of factor V gene (letter). *Thromb Haemost*, 75:522.

Miller, S.A., Dykes, D.D., Polesky, H.F. (1988) *A simple salting out procedure for extracting DNA from human nucleated cells*. *Nucleic Acids Research*., 16 : 3 : 1215

Hurber, S., McMaster, K. J. and Voelkerding, K. V. (2000)Analytical Evaluation of Primer Engineered Multiplex Polymerase Chain Reaction–Restriction Fragment Length Polymorphism for Detection of Factor V Leiden and Prothrombin G20210A. *Journal of Molecular Diagnostics.,* 2:153-157

Bertina, R.M. (1997)Factor V Leiden and other coagulation factor mutations affecting thrombotic risk. *Clin Chem*., 43:1678–1683.

Saxena, R., Mohanty, S., Srivastava, A., Choudhry, V.P., Kotwal, J.(1999) Pathogenetic factors underlying juvenile deep vein thrombosis (DVT) in *Indians. Eur J Haematol*.. 63:26-8.

Ghosh, K., Shetty, S., Madkaikar, M. (2001) Venous thromboembolism in young patients from western Indian study. *Clin Appl Thromb Hemost*.,7:158–65.

Pawar, A.R., Shetty, S., Ghosh, K., Mohanty, D. (2001) How old is factor V Leiden mutation? *Thromb Hemost.,* 86:1591-2 .

Koshy, A., Jeyakumari, M. (2006) Factor V Leiden is not commonly associated with idiopathic portal vein thrombosis in southern *India. Indian J Gastroenterol*, 25:140-2.