ORIGINAL ARTICLE

A CROSS SECTIONAL STUDY TO ASSESS THE SPECTRUM OF INHERITED THROMBOPHILIA IN ADULT (20 TO 50 YEARS OF AGE)

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ABSTRACT

OBJECTIVES: The aims and objectives of the study were to identify the patients with hereditary predisposition for thromboembolism and to assess the distribution of natural anticoagulants deficient in such patients.

METHODS: It was a descriptive cross sectional study. A screening test ProC Global was carried out to detect deficiency of protein C or S and then susceptibility of protein C and protein S assays were carried out on the positive cases. The level of antithrombin and the screening test for factor V Leiden were carried out separately.

RESULTS: A total of 264 patients were referred for ProC Global out of which 25 (39.0 %) were positive. The protein C was deficient in 3 patients detected by protein C assay and no patient was deficient in protein S. Antithrombin deficiency was detected in 16 out of 190 (8.4 %) patients and screening test for factor V Leiden was positive in three out of 30 (10 %) cases.

CONCLUSION: Inherited thrombophilia is common in patients having a positive history of arterial or venous thrombosis.

KEY WORDS: Inherited Thrombophillia, ProC Global, Anti Thrombin, Factor V Leiden

INTRODUCTION

Thrombophilia is a term used to describe a group of conditions in which there is an increased tendency of thrombosis in the venous or arterial system. Thrombotic events are increasingly recognized as a significant source of mortality and morbidity. There are many conditions in which this state may occur, some of which are hereditary and others are acquired. Hereditary thrombophilia is defined as genetically determined increased likelihood of thrombosis.[1]

In 1856, Rudolf Virchow proposed that stasis, injury to the vessel wall and abnormalities in the circulating blood are the three primary causes of venous and arterial thrombosis.[2]

Subsequently numerous investigators unveiled the various steps in the haemostatic balance. An emerging paradigm suggest that thromboembolism is involved in one or more genetic defects in conjunction with acquired risk factors such as inactivity, trauma, malignancy, inflammation, pregnancy, oral contraceptive use or autoimmune disease.[3]

The first major breakthrough in understanding of hereditary thrombophilia was made when Egberg in 1965 demonstrated reduced level of antithrombolin in a family whose members suffered from recurrent venous thrombosis. The disorder was inherited in an autosomal dominant pattern. After this discovery multiple studies have shown almost 250 different mutations for anti-thrombin deficiency.[4]

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MATERIAL & METHODS

It was a Descriptive cross sectional study conducted at Haematology Department of AFIP, Rawalpindi. Non Probability sampling technique was adopted for the referred patients (to AFIP), requiring thrombophilia screening test. The duration of study was from March 2010 to September 2010.

The Aims and Objectives of the study were to identify patients with hereditary predisposition for thromboembolism and to assess frequency distribution of deficiencies in natural anticoagulants deficiencies in patients presenting with thromboembolism.

The Inclusion Criteria comprises of the patients having a history of thrombosis at young age (<50 years of age), Deep venous thrombosis (DVT), Pulmonary embolism (PE), Oral contraceptive use or hormone use, Obesity, Recurrent miscarriages, Stillbirth and Ischemic heart disease.

The old age patients having previously confirmed abnormality in coagulation pathways, malignancy, history of Oral and Parenteral anticoagulant (Warfarin, heparin) intake in the last 6 months, immobility, trauma, and surgery were excluded from

the study.
An informed consent along with the Patients clinical history and examination were the prerequisites of study. All the gathered information was recorded on specially designed proforma. Afterwards 2.5 cc venous blood sample was collected from the selected patient in Trisodium citrate anticoagulant. ProC Global screening was done on all the anticoagulated blood samples. Screening test ProC Global was carried out to detect deficiency of protein C or S. Protein C and S assays were carried out on the positive cases. The level of antithrombin and the screening test for factor V Leiden were carried out separately.
The assessment of Pro C global was done by an Incubation of the plasma with the protein C activator (Venom of Agkistrodon Conportrix). Dade Behring (Siemens KIT) was used for Pro C Global. A contact phase activator causes activation of endogenous protein C and of the intrinsic coagulation cascade.
Coagulation is triggered by the addition of calcium ions. The activated protein C – in conjunction with endogenous protein S inactivates pro coagulatory cofactors VIII-a and V-a. This delays clot formations. The time taken for a clot to from is determine (PCAT = Protein C activity dependent clotting time. In plasmas with a diminished capacity of the protein C system. The coagulation time is less markedly prolonged. The normal control ratio is > 0.80 and normal standard ratio is >0.40.
The Anti thrombin in the sample is converted by heparin into an immediate inhibitor and inactivates the thrombin present. The residual thrombin content is determined in a kinetic test measuring the increasing in absorbance at 405 nm. Normal value of anti thrombin is 75-125
The sample is pre-diluted with factor V deficient plasma. Addition of activator and APTT reagents initially cause the activation of protein C, S and the intrinsic pathway. Coagulation is triggered by the addition of Ca^{+} ion. Activated protein C & S in activates the F- VIII (a) ultimately causing a delay in clot formation. Presence of Factor - V Leiden will lead to a shortening of the clotting time. The normal value of factor V Leiden is >0.8.

RESULTS

The data collected in the form of variables were analyzed by using the SPSS version14.0. The statistical analysis was done by detecting the Frequencies and Percentages to describe the pattern of deficiencies for various anticoagulants.
A total of 264 patients were referred for ProC Global out of which 22 (39.0 %) were positive. The protein C was deficient in 03(10%) patients detected by protein C assay and no patient was deficient in protein S. Anti thrombin deficiency was detected in 16 (8.4 %) patients and screening test for factor V Leiden was positive in 03(10 %) cases. The results of the study are tabulated in Table 1. Table 2 describes the distribution of study population based upon the gender.

TABLE 1: Distribution of Various Natural Anti Coagulants Deficient (n=264)

Sr. No.	Tests	Total No. of patients (n)	Positive Cases (n)	Percentage (%)
1.	Pro c Global	64	25	39.0 %
2.	Protein C	29	03	10 %
3.	Protein S	27	-	–
4.	Anti-thrombin	190	16	8.4 %
5.	Factor V - Leiden	30	03	10 %

TABLE 2: Demographic Data based upon gender distribution of 22 positive cases

Sr. No.	Tests	Total No. of patients (n)	Male		Female		Total	
			n	%	n	%	n	%
1.	Protein C	29	02	6.86	01	3.4	03	10.34
2.	Protein S	27	-	-	-	-	-	-
3.	Anti-thrombin	190	07	3.68	09	4.73	16	8.42
4.	Factor V Leiden	30	01	3.3	02	6.6	03	9.9

DISCUSSION

In Pakistan only few studies have been done to find the spectrum of genetic defects in natural anticoagulants and their frequency in patients presenting with thromboembolism.[13] In the light of this situation, this study has been planned at Haematology department, to find the frequency of deficiencies in various natural anticoagulants leading to hereditary thrombophilia.

The current study results have shown that Protein C and Factor V Leiden are the commonly deficient anticoagulant responsible for inherited thrombophillia. This is in favour of the study carried out by Griffins et al and Murin et al, who found that protein C deficiency predisposes to thrombosis [16]. While second in sequence was Protein S deficiency which was demonstrated in families with thrombosis. This finding however differs from the current study results.[5] All of these proteins, anti-thrombin, protein C and protein S play a role in the down regulation of coagulation. Deficiencies of these proteins result in an increased generation of thrombin and a predisposition to thrombosis.[6]

In 1993, Dahlback et al described a family from Sweden who had history of thrombosis in males and females throughout several generations and showed an autosomal dominant pattern of inheritance. They observed a significant prolongation of the activated partial thromboplastin time (APTT) in the normal plasma following the addition of activated protein C (APC).[7]

APC inactivates Factor Va and factor VIII-a, thereby decreasing available thrombin. In contrast plasma from the affected family showed a lack of significant prolongation of APTT. The researchers concluded that there was an abnormality in the protein C and S regulatory system. This abnormality was identified as a single amino acid substitution in one of the substrate protein for APC.[7]

In 1994, Lane et al described a defect in the factor V gene that makes it less susceptible to inactivation by APC. Further studies have shown that most patients with APC resistance have a factor V allele that is resistant to the proteolytic effect of protein C. A transition (guanine to adenine) at nucleotide (1691) leading to replacement of arginine by glutamine at position 506. This study was done by investigators from Leiden in Netherland.[8] It is now recognized as the most common cause of heritable thrombophilia in Caucasians. The prevalence of factor V Leiden is approximately 3 to 5% among Caucasians. Whereas it is very rare in native African and Asian population.[9,13]

Deep venous thrombosis (DVT) is the most common clinical presentation of thrombophilia associated with factor V Leiden. The mutation is found in approximately 20% of unselected patients with DVT and 40 to 60% of selected patients referred to coagulation centres for evaluation.[9]

The studies by Goforth et al and Papa et al concluded that Factor V Leiden mutation predisposes to thromboembolism genesis [14,15].

Prothrombin is the precursor molecule of thrombin which activates factor V and VII and converts fibrinogen to fibrin.[10] In 1996, Port etal sequenced the prothrombin gene from 28 families with un-explained thrombophilia and identified a guanine to adenine translocation at nucleotide position 20 to 10 in the 3-untranslated region of the gene that is associated with an increase risk for venous thrombosis.[11] The G20 to 210A allele of the prothrombin gene has been confirmed to be one of the most prevalent genetic factor associated with venous thrombosis.[12] It is present in 5 – 6 % of unselected patient with venous thrombosis. There are a number of other genetic defects or isolated deficiencies that have been implicated in contributing to the risk of thrombosis in families with thrombophilia.

A study report by Morris et al concluded that use of oral anticoagulants can beneficial for management of inherited thrombophilia [17]. Therefore the early and proper diagnosis will be the necessary tool to reduce the morbidity and mortality rates in various age groups [18,19].

CONCLUSION

Inherited thrombophilia is common in patients having a positive history of venous thrombosis. Thus, the early detection/ diagnosis may be helpful in starting the accurate treatment ultimately resulting in reducing the morbidity and mortality rates in underdeveloped countries like one of ours.

REFERENCE

1. Hoppe C, Matsunaga A. Paediatric hrombosis. Paediatr Clin North America. 2002; 49: 125.
2. Goodnight SH, Griffin JH. Hereditary Thrombophilia. Williams Haematol. 2006; 7th Ed. 1697-710.
3. Rosendaal FR. Venous Thrombosis, a multicausal disease. Lancet. 1999; 353:1167-73.
4. Khan S, Dickerman JD. Hereditary Thrombophilia. Thrombosis 2006; 4:15.
5. Lane DA, Mannuci PM, Baur KA, Bertina RM, Bochkov N, Boulijenkov V, et al. Inherited Thrombophilia. Thromb Haemost. 1996; 75: 824-34.

6. Khalid S, Sajid S, Adil N, Khurshid N. Frequency of Hereditary Thrombophilia: An AKUH experience. JPMA. 2004; 54: 427-9.
7. Dahlback B, Hillarp A, Frutos PB, Zoller B. Thrombophilia as a multigenic disease. J Haematol. 1999; 84:59-70.
8. Seligsohn URI, Lubetsky A. Genetic susceptibility to venous thrombosis. N Eng J Med. 2001; 344: 1222-31.
9. Greer JP, Foerster J, Lukens JN, Rogers GM, Frixos Paraskevas, et al. Disorder of Haemostasis and Coagulation. Wintrobe's Clin Haematol. 2004; 11th Ed: 1784-800.
10. Jackson BR, Holmes K, Phansalker A, Rodgers GM. Testing for hereditary thrombophilia: a retrospective analysis of testing referred to a national laboratory. BMC Clin Pathol. 2008; 8: 3.
11. Stefano VD, Rossi, Paciaroni, Leone G. Screening for inherited thrombophilia: Indications and therapeutic implications. Haematol. 2002; 87: 1095-1108.
12. Junaid A, Ahmed S, Anwar M, Ali W, Din N, Ayyub M, et al. Factor V Leiden in young patients with thrombophilia. JCPSP. 2004; 14(11): 698-9.
13. Hussain N, Shamsi T, Soomro N. Frequency of thrombophilia in patients with adverse pregnancy outcome. JPMA. 2005; 55: 245-7.
14. Papa A, Danese S, Grillo A, Gasbarrini G, Gasbarrini A. Inherited thrombophilia in inflammatory bowel disease. Am J Gastroentrol. 2003; 98(6): 1247-51.
15. Goforth RL, Rennke H, Sethi S. Renal vascular stenosis is associated with inherited thrombophilia. Kidney Internal. 2006; 70: 743-50.
16. Murin S, Gregory P, Arroliga AC, Richard A. Hereditary thrombophilia and venous thromboembolism. Am J Respir Crit Care Med. 1998; 158 (5): 1369-73.
17. Morris JG, Singh S, Fischer M. Testing for inherited thrombophilia in arterial stroke. Stroke 2010; 41:2985-90.
18. Stefano VD, Rossi E, Paciarini K, Leone G. Screening for inherited thrombophilia, indication and therapeutic. Haematol. 2002; 87(10): 1095-1108.
19. Kupferminie MJ. Thrombophilia and pregnancy. Reprod Biol Endocrinol. 2003; 1: 111.