Research Article

Common thrombophilia markers in young patients with primary arterial and venous thrombosis

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ABSTRACT

Background: The pathogenesis of vascular thrombotic event involves an interaction of multiple genetic and environmental factors. Genetic factors include activated protein C resistance, deficiency of natural anticoagulants protein C, protein S, factor V and antithrombin III while acquired cause include presence of antiphospholipid antibody. This study was carried out to establish the role of basic panel of thrombophilia in diagnosing the patients with primary thrombophilia.

Methods: A total of 121 consecutive patients with history suggestive of arterial or venous thrombosis were included. History of risk factors including smoking, hypertension, hyperlipidemia, diabetes mellitus and family history of thrombosis were elicited. The initial tests included CBC, PT, APTT, LFT, blood sugar and lipid profile. Functional assay on thrombophilia markers were performed by means of clotting based method.

Results: A total of 121 patients were studied, out of which 63 were males and 58 females. The mean age range was 15-40 years. No abnormality was detected in 75 samples, while 46 samples (38%) were positive for one or more thrombophilia markers, of which 6 had more than one thrombophilia marker. Abnormality of a single thrombophilia factor was found in 40 patients, in which two patients were also positive for lupus anticoagulant.

Conclusions: An association was found between low levels of protein C, protein S and factor V and a thrombotic event. Screening of these patients using a panel of thromophilia markers can provide valuable information in primary diagnosis of inherited deficiency even in the absence of high end molecular/mutational studies.

Keywords: Thrombosis, Protein C, Protein S, Antithrombin III

INTRODUCTION

Thrombophilia is characterized by clinical tendency to arterial or venous thrombosis that predisposes to thromboembolic disease. In 1856, Rudolf Virchow first described the triad of stasis of blood flow, vascular endothelial damage and hypercoagubility of blood. It was later used to describe the etiology of thrombosis.[1]

The term thrombophilia was introduced by Egeberg in 1975.[2] Thrombosis results due to the interaction of multiple genetic as well as acquired factors. Genetic factors include activated protein C resistance, deficiency of natural anticoagulants like protein C, protein S, antithrombin III. In hereditary thrombophilia, there is as an enhanced inherited tendency to form intravascular thrombi, which maybe arterial or venous that characteristically occurs in young age (before 45 years) and tends to recur.[3]

Among the acquired causes, commonest is the presence of anti-phospholipid antibodies.[4] The overall incidence of thrombophilia is low in general population worldwide, hence routine testing is not performed.[2] However, in view of paucity of studies on Asian Indians, this study was embarked upon to determine the prevalence of the

common thrombophilia markers in the young patients of northern India presenting with primary arterial or venous thrombosis. It was aimed to establish a basic panel of thrombophilia markers that could be used as a screening tool.

METHODS

The present study was conducted in the department of pathology from January 2013 to October 2015. A total of 121 consecutive patients of all age groups and both sexes with history suggestive of arterial or venous thrombosis were included.

The inclusion criteria for the patients were that they should present one or more of the following:

- Age <45 years at onset of thrombotic episode;
- Absence of any obvious cause of thrombotic event,
- Multiple events of thrombosis,
- Thrombosis at unusual site,
- More than one episode of thrombosis irrespective of age. Blood samples were collected in all the patients after 10-12 weeks of the acute episode.

The patients were discontinued from any oral anticoagulants and started on low molecular weight (LMW) heparin for at least 3 weeks before drawing the sample for testing. Cases not meeting the above criteria and those with secondary thrombophilia were excluded.

For all the patients detailed clinical history regarding smoking, diabetes, hypertension, hyperlipidemia and family history of thrombosis was elicited. History related to previous major surgery, prior myocardial infarction, past major trauma, or other serious medical illness was also taken. The occurrence of thrombotic event was confirmed by investigations like duplex scanning, MR angiography, ECHO etc.

Among the various initial tests conducted were complete blood count (CBC), prothrombin time (PT), activated partial thromboplastin time (aPTT) and lupus anticoagulant. The biochemical tests carried out were blood sugar, lipid profile, and liver function tests.

Blood collected in blue Vaccutainer tubes containing 3.2% sodium citrate (nine volumes of blood to one volume of anticoagulant) and centrifuged at 2500 g for 15 minutes at room temperature and again at 4 °C to remove platelets. Functional assay was performed on thrombophilia markers protein C, protein S, antithrombin III and factor V (APC- resistance).

The tests were carried out by means of clotting based method on the equipment IL 9000. All tests were performed in accordance with the product manual. The mean value and normal range was taken according to the inserts supplied with the product kit as Protein C (60-

120%); Protein S (60-120%), Antithrombin III (83-128%) and Factor V (APC-R:2.18-3.38). Due to budgetary constraints, testing of other thrombophilia markers such as homocysteine, factor V leiden could not be done.

RESULTS

A total of 121 patients were studied, out of which 63 were males and 58 females. The mean age range was 15-40 years (Table 1). Common clinical presentation of the patients included i) Deep venous thrombosis ii) Gangrene foot iii) Post-partum seizure disorder with no previous history of seizures iv) Embolic stroke v) Headache diagnosed as multiple venous thrombosis of cerebral veins vi) Myocardial infarction.

Table 1: Age and sex distribution of patients.

Age (years)	No. of patients	Male	Female
<15	14	11	03
15-40	85	37	48
>40	22	15	7

No abnormality was detected in 75 samples, while 46 samples (38%) were positive for one or more thrombophilia markers, of which 6 had more than one thrombophilia marker. Abnormality of a single thrombophilia factor was found in 40 patients, in which two patients were also positive for lupus anticoagulant.

Table 2: Classification of patients into severe or mild deficiency.

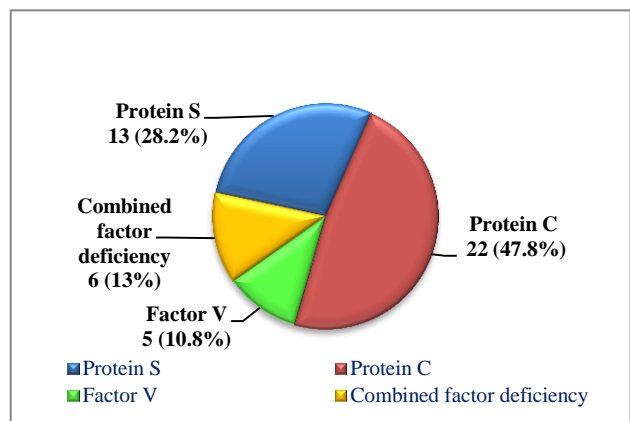| Factors	≤40 units (severe)	40-60 units (mild)	60-120 units (normal)
Number of patients			
Protein C	10	18	68
Protein S	07	11	84

Figure 1: Spectrum of factor deficiency 46 out of 121.

Abnormality of single factor was mainly deficiency of Protein C in 22 patients (47.8%) followed by Protein S

deficiency in 13 patients (28.2%). Factor V resistance (APC-R) was found the least, only in 5 (10.8%) of the positive samples (Figure 1). Anti-thrombin III was found to be normal in all patients.

Patients were classified on the basis of levels of protein C and protein S into group of severe deficiency or mild deficiency or with normal levels of these anticoagulants. Ten patients were found to have severe deficiency of protein C and 18 had mild deficiency. In case of protein S, seven patients were categorized as having severe deficiency and 11 as mild deficiency of protein S (Table 2).

DISCUSSION

Inherited thrombophilia is defined as deficiency of natural anticoagulants protein C, protein S and activated protein C resistance leading to intravascular thrombi characteristically in young age group (<40 years age).[3] In our study the patients presented in the age group 15-40 years. In a study conducted by Mishra et al a similar average age for first thrombotic event was found to be 36 years.[4]

An association was found between low levels of protein C or S or resistance to factor V and the thrombotic event. Out of the total patients 38% were found to be positive for one or more thrombophilia markers similar to some of the studies done on Indian population.[5]

Factor V resistance was found in only few patients (5/46 positive cases) which is in agreement with the study of wolf et al.[6] However, some recent studies have reported a relatively higher frequency of APC-R in Indian population.[7-9] In a study by Khare et al, the prevalence of factor V Leiden was significantly higher in MI cases.[10] APC-R in majority of cases is because of a mutation in the factor V gene, labelled factor V Leiden.[11] The limitations of this study include the lack of DNA studies for detecting factor V Leiden, which is the commonest cause of APC-R. There is also a need to look for other mutations that could lead to APC-R in Indians.[2]

In our study, Antithrombin III was found normal in all patients. Antithrombin deficiency is significantly not related to increased risk of thrombosis. A high risk for arterial thrombosis conferred by protein S or protein C but not antithrombin deficiency has also been reported earlier.[3]

We also found certain rare sites of thrombosis like multiple cerebral venous thrombosis in our study. Deficiency of severe grade correlated with higher frequency of recurrence and severity of thrombotic episode in our patients. The deficiency of multiple factors in some patients, although low, highlights the relevance of multiple factors in combination as the cause of thrombotic event. Thrombophilia screening is extremely expensive for a developing country like India. Careful and selective thrombophilia testing should be done in all patients in whom the results would affect their medical management or provide useful data for the health care.

These tests should not be performed routinely and should be done after detailed clinical as well as family history of patients. Furthermore, these tests must not be done during the acute stage because the acute phase of thrombosis and anticoagulant therapy considerably affect the results of many phenotypic assays making interpretation of the results difficult and unreliable.

CONCLUSION

Screening of these patients using a panel of thrombophilia markers can provide valuable information in primary diagnosis of inherited deficiency even in the absence of high end molecular/mutational studies which is not available in most of the set ups. Hence, prudent selection of tests is of utmost importance. Moreover, there is a need of more awareness among the clinicians so as to increase the number of cases sent for investigation. Pooling of patients can be done to reduce wastage of the kits and thus the costs involved.

REFERENCES

1. Kumar DR, Hanlin E, Glurich I, Mazza JJ, Yale SH. Virchow's contribution to the understanding of thrombosis and cellular biology. Clin Med Res. 2010;8(3-4):168-72.
2. Caprini JA, Goldshteyn S, Glase CJ, Hathaway K. Thrombophilia testing in patients with venous thrombosis. Eur J Vascular and Endovascular Surgery. 2005;30(5):550-5.
3. Soare AM, Popa C. Deficiencies of Proteins C, S and Antithrombin and Activated Protein C Resistance– Their Involvement in the Occurrence of Arterial Thromboses. J Med Life. 2010;3(4):412-5.
4. Mishra MN, Bedi VS. Prevalence of common thrombophilia markers and risk factors in indian patients with primary venous thrombosis. Sao Paulo Med J. 2010;128(5):263-7.
5. Mishra MN, Kalra R, Rohatgi S. Clinical profile, common thrombophilia markers and risk factors in 85 young indian patients with arterial thrombosis. Sao Paulo Med J. 2013;131(6):384-8.
6. Wolf M, Boyer-Neumann C, Martinoli JL, Leroy-Matheron C, Amiral J, Meyer D, et al. A new functional assay for human protein S activity using activated factor V as substrate. Thromb Haemost. 1989;62(4):1144-5.
7. Herrmann FH, Salazar-Sanchez L, Schröder W. Prevalence of molecular risk factors FV Leiden, FV HR2, FII 20210G>A and MTHFR677C>T in

different populations and ethnic groups of Germany, Costa Rica and India. IJHG. 2001;1(1):33-9.

8. Bhattacharyya M, Kannan M, Chaudhry VP, Saxena R. Venous thrombosis: prevalence of prothrombotic defects in north Indian population. Indian J Pathol Microbiol. 2003;46(4):621-4.

9. Saxena R, Mohanty S, Srivastava A, Choudhry VP, Kotwal J. Pathogenetic factors underlying juvenile deep vein thrombosis (DVT) in Indians. Eur J Haematol. 1999;63(1):26-8.

10. Khare A, Ghosh K, Shetty S, Kulkarni B, Mohanty D. Combination of thrombophilia markers in acute myocardial infarction of the young. Indian J Med Sci. 2004;58(9):381-8.

11. Anderson FA, Spencer FA. Risk Factors for Venous Thromboembolism. Circulation. 2003;107:I9-16.