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Role of Antithrombin III, Plasminogen, Protein C and Protein S in Deep Vein Thrombosis in Indian Population

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ABSTRACT

Deep vein thrombosis is a common condition which is often underdiagnosed. A deficiency of antithrombin III, plasminogen, protein C or protein S, may be associated with a prothrombotic state and can predispose patients to an increased risk of developing deep vein thrombosis. Deficiencies of these anticoagulant proteins may be acquired or congenital. Congenital anticoagulant protein deficiency should be suspected in patients with recurrent deep vein thrombosis without identifiable risk factors. The recognition of this entity is important to prevent development of complications like pulmonary thromboembolism which may prove to be fatal. Role of anticoagulant protein deficiencies as a cause of deep vein thrombosis is scantly studied in the Indian population. The aim of this study is to determine the role of these anticoagulant protein deficiencies as a cause of deep vein thrombosis.

Key Words: Deep vein thrombosis, DVT, Venous thromboembolism (VTE), antithrombin III, plasminogen, protein C, protein S.

INTRODUCTION

Venous thromboembolism (VTE) is a common and potentially life-threatening condition. It continues to be underdiagnosed and undertreated. Awareness among Indians regarding this potentially life-threatening disease is low. The exact incidence of venous thrombosis is difficult to define because of the often-silent nature (80%) of the condition. Incidence of venous thrombosis in Asia, India & South Asian countries is 6-75%. [1] Venous thrombosis is more common in Europe and USA than in Asia and Africa. The difference in incidence has been attributed to differences in genes, diet, fibrinolytic activity and climate. [2-4]

Hospitalized patients are especially at an increased risk for venous thromboembolism as most of them have multiple risk factors. It has been found in autopsy studies that the incidence of VTE in hospitalized patients is as high as 34.7% with the incidence of fatal pulmonary thromboembolism in 9.4%. [5]

Venous thromboembolism has clinical manifestations that range from thrombosis in the deep veins of legs, the iliac and abdominal major vessels to ultimately pulmonary embolism. Venous thromboembolism and deep vein thrombosis rarely occur in the absence of risk factors. Classically acquired risk factors for developing venous thrombosis include advanced age, prolonged immobilization, surgery, fractures, use of oral contraceptive pills, hormone replacement therapy, pregnancy, puerperium, cancer and antiphospholipid antibody syndrome. [6]

Venous thrombosis is related to three pathologic factors commonly known as "Virchow's triad" which includes:

1. Vessel wall damage.

2. Blood hypercoagulability
3. Blood stasis

Venous thrombi are made up of fibrin, red blood cells, platelets, and leucocytes. These thrombi start in areas of slow blood flow or turbulent blood flow such as large venous sinuses or venous valve cusps and also in areas of direct venous trauma. Activation of the coagulation pathway is the crucial step in the initial formation of venous thrombi. It occurs due to local injury or remote release of mediators. Activation of the pathway alone is inadequate in formation of a venous thrombus as inhibitors of thrombosis such as antithrombin, thrombomodulin-protein C and S, tissue factor pathway inhibitor (TFPI) along with the fibrinolytic pathway will clear the clot. Therefore, persistent activation due to endothelial stimulation along with poor blood flow failing to clear the activated factors, results in an imbalance in the pro and anti-thrombotic pathways which ultimately leads to progression of the thrombus.

Surgical patients have all the three Virchow's factors present in the peri-operative period. They have venous stasis due to immobilization and surgical positioning. Direct venous injury or remote release of mediators of coagulation due to tissue trauma also increases the risk of venous thrombosis. The risk factors for a surgical patient developing venous thromboembolism have been extensively studied and the important determinants appear to be age, type of surgery, length of procedure, and duration of immobilization. [7]

Genetic abnormalities increasing the risk of thrombotic events have been known since several decades. They include deficiencies of the natural anticoagulants like antithrombin III, protein C, and protein S. Other factors include factor V Leiden, prothrombin G20210A, high levels of factors VIII, IX, XI, homocysteine, and fibrinogen. [8-14]

Antithrombin is a naturally occurring anticoagulant that inactivates serine proteases such as thrombin, and clotting factors IXa, Xa, XIa, and XIIa. Patients with a deficiency of antithrombin are at risk for both arterial and venous thrombosis.

Plasminogen is synthesized in the liver and is present in other cells and in extravascular space of most tissues. Plasminogen is converted to a proteolytic enzyme, plasmin, by plasminogen activators including tissue-plasminogen activator (tPA) and urokinase-plasminogen activator (uPA). The main action of plasmin is to break down fibrin through a series of proteolytic cleavages. Defective fibrinolysis has been associated with increased risk of deep vein thrombosis.

Protein C, a vitamin K dependent protein, is synthesized in the liver and contributes to the inactivation of factor VIII. Deficiency of protein C may be associated with severe thrombotic events.

Protein S, a vitamin K-dependent glycoprotein, is synthesized by the liver and acts as the principal cofactor of protein C. Deficiency of protein S increases the risk of venous thrombosis.

In many cases of Deep vein thrombosis, no predisposing risk factors have been identified. Anticoagulant Protein deficiencies may be a cause in such idiopathic cases.

Our study shall endeavour to determine the levels of anticoagulant proteins like protein C, protein S, Antithrombin III and plasminogen in cases of deep vein thrombosis and their correlation with deep vein thrombosis.

MATERIALS AND METHODS

It was a case control study conducted in Department of Pathology and Department of Surgery, in a tertiary care hospital in New Delhi.

This study was carried out on 60 cases who presented to the surgery department with swelling and crampy pain during walking. On examination, along with assisted duplex scanning they were diagnosed with deep vein thrombosis. These

patients were not receiving any anticoagulant drugs or any other drugs which could interfere with coagulation pathways. These patients also did not have any prior history of any medical illness or any previous surgical history.

Blood from sixty age and sex matched healthy donors from blood bank was taken as controls for comparison. All donors who were receiving any drug which interferes with coagulation pathways were excluded from the study.

2ml of blood sample was taken in EDTA vial. It was centrifuged and then it was run on Automated Nephelometer for the measurement of Antithrombin III and plasminogen.

3ml sample was taken in 3.8% sodium citrate solution in a ratio of 9:1. It was centrifuged at 2500 rpm for 15 minutes and then it was run on the Fully Automated Coagulometer for Protein C and Protein S measurement.

Statistical analysis -

Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD and median. Normality of data was tested by Kolmogorov-Smirnov test. If the normality was rejected, then non-parametric test was used.

Statistical tests were applied as follows-

1. Quantitative variables were compared using Unpaired t-test/Mann-Whitney Test (when the data sets were not normally distributed) between the two groups.
2. Qualitative variables were correlated using Chi-Square test /Fisher's exact test.
3. Univariate and multivariate logistic regression was used to assess the association of presence of disease with various parameters.

A p value of <0.05 was considered statistically significant. The data was entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0.

RESULTS

Out of all the 60 cases, antithrombin III deficiency was found in 18 cases (30%). In 42 cases the levels of antithrombin III were found to be within the normal range. In the control group, 6 controls (10%) were found to have deficiency of antithrombin III and it was normal in the remaining 54 controls. The correlation was not significant (P value = 0.006).

Table 1 - The distribution of antithrombin III deficiency in both the groups

		Group		Total	P value
		Case	Control		
ATIII distribution	Not deficient	42 (70.00%)	54 (90.00%)	96 (80.00%)	0.006
	Deficient	18 (30.00%)	6 (10.00%)	24 (20.00%)	
Total		60 (100.00%)	60 (100.00%)	120 (100.00%)	

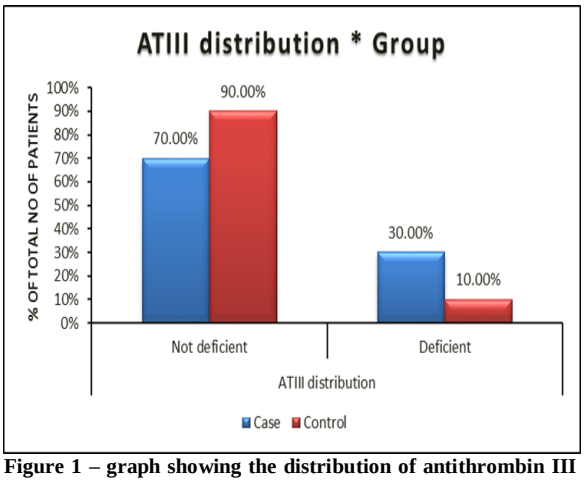

Figure 1 – graph showing the distribution of antithrombin III deficiency in both the groups

Out of the total 60 cases, plasminogen deficiency was found in 5 cases (8.33%). In 55 cases (91.67%) the levels of plasminogen were found to be within the normal range.

In the control group, all the controls had normal levels of plasminogen. The correlation was not significant (P value = 0.057).

Table 2 - The distribution of plasminogen deficiency in both the groups

		Group		Total	P value
		Case	Control		
Plas distribution	Not deficient	55 (91.67%)	60 (100.00%)	115 (95.83%)	0.057
	Deficient	5 (8.33%)	0 (0.00%)	5 (4.17%)	
Total		60 (100.00%)	60 (100.00%)	120 (100.00%)	

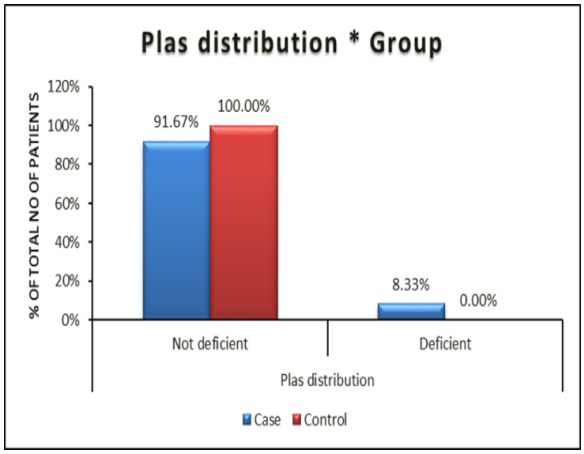

Figure 2 – graph showing the distribution of plasminogen deficiency in both the groups

Out of all the 60 cases, protein C deficiency was found in 13 cases (21.67%). In the remaining 47 cases (78.33%) the levels of protein C were found to be within the normal range.

In the control group, all the controls had normal levels of protein C. The correlation was found to be statistically significant (P value = 0.0001).

Table 3 - The distribution of protein C deficiency in both the groups

		Group		Total	P value
		Case	Control		
Prot C distribution	Not deficient	47 (78.33%)	60 (100.00%)	107 (89.17%)	0.0001
	Deficient	13 (21.67%)	0 (0.00%)	13 (10.83%)	
Total		60 (100.00%)	60 (100.00%)	120 (100.00%)	

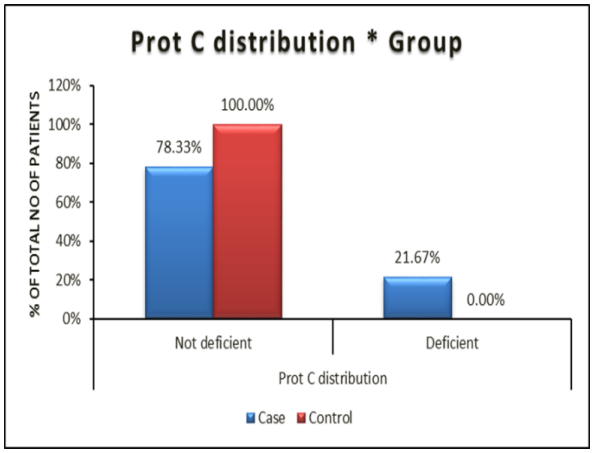

Figure 3 – graph showing the distribution of protein C deficiency in both the groups

Protein S deficiency was found in 18 cases (30%) out of the total 60 cases. In 42 (70%) cases the levels of protein S were found to be within the normal range.

In the control group, 3 controls (5%) were found to have deficiency of protein S and it was normal in the remaining 57 controls (95%). The correlation was found to be statistically significant (P value = 0.001).

Table 4 - The distribution of protein S deficiency in both the groups

		Group		Total	P value
		Case	Control		
Prot S distribution	Not deficient	42 (70.00%)	57 (95.00%)	99 (82.50%)	0.001
	Deficient	18 (30.00%)	3 (5.00%)	21 (17.50%)	
Total		60 (100.00%)	60 (100.00%)	120 (100.00%)	

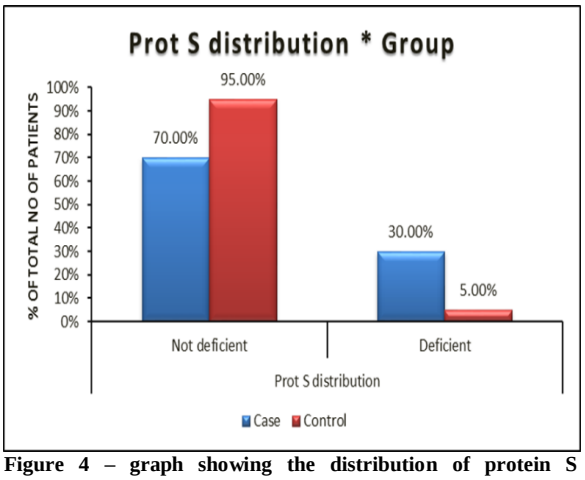

Figure 4 – graph showing the distribution of protein S deficiency in both the groups

DISCUSSION

Deficiency of natural anticoagulants can either be inherited or may occur during certain life events. Genes for the natural anticoagulants, are inherited from parents. People born with deficiencies of one of the natural anticoagulants inherit one abnormal gene from either their mother or father. Rarely people can inherit abnormal genes from both parents; this often results in severe clotting problems that are diagnosed early in infancy. People who have inherited normal levels of the natural anticoagulants may develop deficiencies in certain situations, such as pregnancy, liver disease, severe infection or other illness, vitamin K deficiency, and certain medications like estrogen, heparin, and warfarin.

Few studies which have been done to assess the role of natural anticoagulants

in venous thrombotic cases showed that deficiencies of natural anticoagulants are important risk factors which can lead to venous thrombosis.

CONCLUSION AND RECOMMENDATIONS

Natural anticoagulant deficiencies are rare and are either inherited at birth or acquired sometime during life. Natural anticoagulant deficiencies are one of many conditions that can increase the risk for developing deep vein thrombosis or venous thromboembolism.

The present study was a case control study which was done on 60 deep vein thrombosis cases who presented to surgery department at Safdarjung hospital, New Delhi. 60 age and sex matched controls were taken from blood bank. An attempt was made to find out the correlation between antithrombin III, plasminogen, protein C and protein S in the patients presenting with deep vein thrombosis.

We found significantly lower levels of protein C and protein S in the cases of deep vein thrombosis as compared with the levels found in controls. In our study, the difference in the levels of antithrombin III and plasminogen in the patients with deep vein thrombosis was not significantly decreased as compared to the controls. Although other studies that have been done in the past have also found significantly lower levels of antithrombin III and other natural anticoagulants in addition to levels of protein C and protein S. This difference may be due to differences in the genetic and environmental factors of the populations being studied in different studies.

From this study, it was concluded that the deficiencies of natural anticoagulant proteins can contribute to increased risk of deep vein thrombosis in the Indian population.

Hence based on our study we advise that all patients presenting with deep vein thrombosis should be evaluated for deficiencies of natural anticoagulant proteins. Further research is needed to evaluate the beneficial effects of screening programmes in the people who present with deep vein thrombosis to prevent development of recurrent episodes of deep vein thrombosis and other serious complications like pulmonary thrombo-embolism.

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