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Surgery

DEFICIENCY OF PROTEIN C AND PROTEIN S IN PATIENTS OF DEEP VEIN THROMBOSIS IN NORTH INDIA.

KEY WORDS: Deep vein thrombosis, thrombophilia factors, protein C, protein S.

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ABSTRACT
 Deficiency of Protein C and protein S are important thrombophilic factors associated with increased incidence of deep vein thrombosis. The present study was conducted on 50 patients attending surgery OPD and venous disease clinic at Post Graduate Institute of Medical Sciences Rohtak, which were divided in two groups, 40 patients in group A having deep vein thrombosis (DVT) proved on color Doppler, and 10 control patients in group B who were not having any venous disease and the prevalence of Protein C and protein S deficiency was studied. In Group A, there were 17 patients out of 40 (42.5%) who were having decreased Protein C levels while in group B, there was 1 patient out of 10 (10%) who was having decreased Protein C levels. Odds ratio came out to be 7.30. The prevalence of Protein C deficiency was more in Group A patients and it was statistically significant. In Group A, there were 5 patients out of 40 (12.5%) who were having decreased levels of Protein S while in group B, there was no patient who was having decreased levels of Protein S. Odds ratio came out to be 5. The prevalence of Protein S deficiency was more in Group A patients but it was not statistically significant.

INTRODUCTION

Deep vein thrombosis (DVT) is characterized by the formation of thrombus or a blood clot in the lumen of the deep veins^{1,2}. DVT occurs in 20-30% of patients after major surgical operations.² When prophylactic measures are not used, it may occur in 40-50% of patients after major gynaecological and orthopaedic especially pelvic surgeries.^{2,3}

The factors which predispose to venous thromboembolism were initially described by Virchow in 1856 and include a triad of hypercoagulability, endothelial damage or intimal changes and reduced blood flow or stasis.^{1,2} However factors relating to platelet activity and fibrinolytic potential of blood are also associated with DVT.^{1,3} Various thrombophilic factors known to cause DVT are: deficiency of anti-thrombin III, protein C or protein S; antiphospholipid antibody or lupus anticoagulant, factor V Leiden gene defect or activated protein C resistance, dysfibrinogenemia and hyperhomocysteinemia.^{1,4,5}

Protein C, a glycoprotein synthesized in the liver, circulates in the blood as an inactive zymogen. Activated protein C (aPC) exerts its anticoagulant activity primarily through inactivation of coagulation factors Va and VIIIa, which are required for factor Xa activation and thrombin generation.⁶ This may explain, in part, why protein C deficiency appears to be associated primarily with venous thrombosis.

Protein S, a vitamin K-dependent physiological anticoagulant, acts as a nonenzymatic cofactor in the proteolytic degradation of factor Va and factor VIIIa. Decreased (antigen) levels or impaired function (activity) of protein S leads to an increased propensity to venous thrombosis.⁷

So this study was conducted to study the levels of Protein C and Protein S and to calculate their prevalence in patients of DVT in comparison with normal population in northern region of India.

MATERIAL AND METHODS

This non randomized case controlled study was conducted at Post Graduate Institute of Medical Sciences Rohtak. Fifty patients were taken who presented in Venous Disease Clinic and Surgery OPD. The patients were divided into two groups: Group A containing 40 patients of deep vein thrombosis with diagnosis proved by colour Doppler and Group B containing 10 patients who did not have any vascular disorder. In all the patients detailed history was taken along with a complete physical examination.

All the patients were subjected to laboratory investigations, prothrombin time (PT) and index (PTI) and activated partial thromboplastin time (aPTT). Samples for measuring the levels of Protein C and Protein S were drawn prior to the initiation of anticoagulant therapy. Protein C was measured on an ACL 9000 Coagulometer (Instrumentation Laboratories, Lexington, MD, USA) using IL Test ProClot (Instrumentation Laboratory) reagent. Protein S was measured on an ACL 3000 Coagulometer by the method of IL-Test protein S (Instrumentation Laboratory). The IL Test ProClot kit is a functional clotting protein C assay, based on the prolongation of the aPTT in the presence of activated protein C.⁸ The IL Test protein S kit is a functional clot based assay of free protein S. In the study our laboratory normal range for protein C and protein S were 0.60-1.20 U/ml and 0.60-1.20 U/ml respectively.^{9,10}

All the patients of Group A were treated with conventional treatment of deep venous thrombosis by low molecular weight heparin for 7 days followed by oral anticoagulants for 6 months. Dose of heparin and oral anticoagulants was titrated as per activated partial thromboplastin time (aPTT) and prothrombin time and International Normalized Ratio (INR) levels which were kept between 2.5 to 3.5. At the end of the study, data obtained was tabulated, analyzed and subjected to statistical analysis using Chi-square test and Student t-test and a conclusion was drawn to evaluate the prevalence of protein C and S deficiency in both the groups.

OBSERVATIONS AND RESULTS

The age of the patients in Group A ranged from 19 to 65 years (Mean 39.32). While in Group B, age of the patients ranged from 18 to 65 years (mean 40.01). In group A, 23 patients (57.5%) were males and 17 patients (42.5%) were females. The female to male ratio was 0.74:1. In group B, 4 patients (40%) were males while 6 patients were females. In group A, the BMI of the patients ranged from 17.67 to 36.15 (Mean 23.61±3.47) while in group B, the BMI of the patients ranged from 18.49 to 25 (Mean 22.71±2.55). Both the groups were statistically comparable in age, sex and BMI distribution.

In Group A, out of 38 patients with unilateral DVT, there were 25 patients (65.8%) with left leg DVT and 13 patients (34.2%) with right leg DVT. Upper limb was not involved in any patient. Two patients (5.2%) had bilateral DVT. Pain and swelling were the most consistent presenting symptoms with a prevalence of 97.5% and 100% respectively. Ten patients (25%) were having swelling up to knee region while 30 patients (75%) were having swelling extending above knee region.

In Group A, there were 17 patients out of 40 (42.5%) who were having decreased Protein C levels while in group B, there was 1 patient out of 10 (10%) who was having decreased Protein C levels. Odds ratio came out to be 7.30. The prevalence of Protein C deficiency was more in Group A patients and it was statistically significant. In Group A, there were 5 patients out of 40 (12.5%) who were having decreased levels of Protein S while in group B, there was no patient who was having decreased levels of Protein S. Odds ratio came out to be 5. The prevalence of Protein S deficiency was more in Group A patients but it was not statistically significant.

There were 2 out of 10 patients (20%) with DVT in below knee region and 15 out of 30 patients (50%) with DVT extending above knee region who were having Protein C deficiency. Statistically this difference was not significant. There was no patient of protein S deficiency in DVT below knee region and 5 out of 30 patients (16.66%) with DVT extending above knee region were having Protein S deficiency. Statistically this difference was significant (p value- 0.032) showing that Protein S deficiency is associated more with above knee swellings i.e. proximal DVT.

DISCUSSION

The incidence of DVT is approximately 1 million which ranges between 5 and 9 per 10,000 persons per year in general population and the incidence of the VTE (DVT and PE combined) is approximately 14 per 10,000 persons per year.^{11,12} In the literature many studies are available regarding levels and prevalence of Protein C and S in western countries. But there are only few studies in the literature available in Indian population. So this study was conducted to measure the serum Protein C and S in DVT patients in this region of India and to know their prevalence.

In the present study, the mean age of the patients with DVT was 39.32 years while in control group the mean age was 40.01 years. The mean age of patients with DVT in the literature has been reported between 57 to 61.¹³⁻¹⁸ In the present study patients were in younger age group possibly because of regional differences in age related incidence of DVT. In the literature most of the studies from western countries have reported a higher incidence of DVT in female patients as compared to male patients. But the study conducted by Gautam et al in India reported that female to male ratio is only 0.32 indicating that DVT is more common in male patients.¹⁹ In the present study the female to male ratio was 0.74:1. It might be due to increased awareness and early mobility in pregnancy and puerperium due to better antenatal and postnatal care or due to less smoking in females in Indian population. It might also be due to the regional differences in the incidence of the DVT in respect to sex.

Incidence of thrombosis in left leg is more than thrombosis in right leg. The factors responsible are compression of left side iliac vein by left iliac artery, an overdistended bladder, gravid uterus and congenital webs within the veins. In the present study, ratio of left

leg to right leg involvement in DVT ranged from 1.92:1. In various clinical studies conducted, ratio of left to right leg involvement in DVT ranged from 1.2 to 4.3:1.^{20,21} In the present study, commonest presenting symptoms were swelling and pain of the lower limbs presenting in 100% and 97.5% of the patients respectively. Villa et al had reported pain and swelling as the presenting symptoms in 86% and 72% of patients respectively.¹⁶ In a study done by Diamond et al, presenting symptoms included leg pain in 42.6% of patients and leg swelling in 53.4% of patients.¹⁸ There should be high degree of clinical suspicion in patients presenting with pain and swelling of leg along with the presence of any of the predisposing risk factors.

In present study, 25% patients were having swelling up to below knee region (distal DVT) while 75% patients were having swelling in above knee region (Proximal DVT). In studies conducted by Charbonnier et al, Souheil et al and Chan et al, the patients associated with proximal DVT were 77%, 74.4% and 71% respectively.²²⁻²⁴ In the present study, we found Protein C deficiency in 42.5% of Group A as compared to 10% of group B. The odds ratio was 7.30 showing a strong association between the development of DVT and Protein C deficiency. John et al, Frederick et al and Richard et al found prevalence of deficiency of Protein C in 7.1%, 3.2% and 8.2% patients of DVT respectively, showing association between Protein C deficiency and DVT.^{25,26} In Group A, the prevalence of decreased Protein S levels was 12.5% with odds ratio of 5. No patient in group B had deficiency of Protein S. Christiansen et al and Shen et al found prevalence of Protein S deficiency in 5% and 32.9% of patients respectively. Thus showing a moderate correlation between Protein S deficiency and DVT.²⁷

In the present study the higher prevalence rates of Protein C and S deficiency may be due to regional or racial differences showing a high prevalence in Indian population as compared to Western population.

The prevalence of Protein C deficiency in patients with DVT in below knee region was 20% as compared to 50% with above knee region DVT in the present study. The prevalence of Protein C deficiency was more in patients of proximal DVT but this was not significant statistically. The prevalence of Protein S in patients with DVT in above knee region was 16.66% while there was no patient of distal DVT having Protein S deficiency. The difference was statistically significant.

CONCLUSION

The prevalence of deficiency of Protein C in cases of DVT in present study was 42.5% which was statistically significant. The prevalence of Protein C and S deficiency was more in proximal DVT. Hence we suggest the measurements of Protein C and S in all cases of proximal DVT so that necessary measures to correct the levels of Protein C and S, can be taken and these patients may require anticoagulation for a longer period.

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