Original Article

Assessment of Protein C, Protein S, and prothrombin levels in gastrointestinal carcinoma with and without metastasis

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ABSTRACT

Background: A hypercoagulable or prothrombotic state of malignancy with metastasis occurs due to the ability of tumor cells to activate the coagulation system. It has been hypothesized that hypercoagulation contributes to the significant percentage of morbidity in cancer patients due to their role in metastasis. **Materials and Methods:** A total of 80 patients with gastrointestinal carcinoma such as malignant carcinoma rectum, malignant carcinoma esophagus, and malignant carcinoma colon with and without metastasis from Surgical Gastroenterology Department C.S.M. Medical University, Lucknow, UP, India, were studied in order to evaluate the presence and extent of hemostatic abnormalities in case of gastrointestinal carcinoma. **Results:** The average prothrombin time, activated partial thromboplastin time, Protein C, and Protein S in patients of gastrointestinal carcinoma was less as compared with control. The mean level of Protein C and Protein S ranged from 55% to 90% and 48% to 95%, respectively. Out of the total 80 patients, 7 were Protein C deficient and 5 were Protein S deficient. However, three were Protein C and S both deficient. The Protein C level was significantly lower ($P < 0.0001$) in Protein C deficient patients with metastasis compared with patients without metastasis. Similarly, the Protein S level was significantly lower ($P < 0.0001$) in Protein S deficient patients with metastasis as compared with patients without metastasis. The Protein C and S levels were also lower in those who were deficient with metastasis. **Conclusion:** Our study infers that activated Protein C resistance in gastrointestinal carcinoma with metastasis may contribute to thrombotic episodes in these patients. Cancer patients including GI malignancy are at increased risk for the development of thrombotic events that contribute significantly to the morbidity and mortality of malignancy in these patients.

Key words: Activated partial thromboplastin time, Protein C, Protein S, prothrombin time, thrombosis

INTRODUCTION

An increased frequency of thrombosis in patients with gastrointestinal cancer was first documented in 1865.[1] Since that time, great deal of biological data and several experimental studies have confirmed the important relationship between cancer and hemostatic system.[2-5] Cancerous cells can activate the clotting system directly thereby generating thrombin or indirectly by stimulating mononuclear cells to synthesize and express a variety of procoagulants.[1] In fact, tissue factors and cancer procoagulants are expressed in tumor cells, resulting in the activation of clotting factors VII and X.[2,3] Cytokines released from tumor cells activate coagulant activity on monocytes, thrombocytes, and endothelial cells. Fibrin formation occurs in many types of tumor tissues, and the formation of a fibrin matrix appears to promote tumor growth via the promotion of neoangiogenesis, and by shielding tumor cells against attack from immunocompetent cells.[4] Thrombin also functions as a potent promoter of cancer growth and spread via an increase in tumor cell adhesion through fibrin formation and by affecting angiogenesis.[2,5,6] The development of venous thromboembolism is associated with transient risk factors for thrombosis such as surgery or trauma and permanent risk factors such as factor V Leiden, the prothrombin mutation, Protein C and Protein S deficiency, and antithrombin deficiency. The present study

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was to assess the Protein C, Protein S, and Prothrombin levels in gastrointestinal carcinoma. For this we designed the prospective study to evaluate the changes in clotting parameter in patients with malignancy and to examine their correlation with metastasis. Our main focus on specific abnormalities of Hemostasis, which include Protein C, Protein S, prothrombin time (PT), and activated partial thromboplastin time (aPTT) in gastrointestinal carcinoma with and without metastasis. The protein C system comprises the cellular receptors thrombomodulin (TM) and endothelial Protein C receptor (EPCR), and the soluble Protein C and Protein S. Thrombin binds to TM and this complex jointly with protein C bound to EPCR generates activated Protein C (APC). One of the main function of APC in blood coagulation is to regulate the rate of thrombin generation in complex with protein S by inhibiting factor va and viiia through limited proteolytically cleavage site for APC such as factor V Leiden variants render this protein partially resistant to cleavage by APC and clinical result is an increased risk of venous thrombosis. Similarly, heterozygous deficiencies of Protein C and protein S cause a marked increase in risk of venous thrombosis. Cancer is the second most common disease worldwide. Among some of its protean manifestations are endothelial cell injury, hypercoagulability, venous stasis, and thrombosis. Metastasis in cancers is one of the major causes of morbidity and mortality in such patients. The risk of thrombosis with metastasis is well known. Hence antithrombotic factors, if defective, may cause metastasis of clotting factors and a prethrombotic state. Thus a coexistent defect of this nature with neoplasia may potentiate prethrombotic state in cancer and by implication enhancement of process of metastasis.

MATERIALS AND METHODS

A total of 80 patients with gastrointestinal carcinoma such as malignant carcinoma rectum, malignant carcinoma esophagus, and malignant carcinoma colon with and without metastasis from Surgical Gastroenterology Department, C.S.M. Medical University, Lucknow, UP, India, were studied in order to evaluate the presence and extent of hemostatic abnormalities in case of gastrointestinal carcinoma. Patients with cardiovascular diseases, diabetes, human immunodeficiency virus (HIV) or any infectious disease, previous malignancy or previous thromboembolic events were excluded from the study. In all the patients Hemostasis markers associated with extrinsic, intrinsic pathway as well as Protein C, Protein S estimation was evaluated prior to surgical intervention. Nearly 5 ml blood was collected from patients in citrated vial (ratio 1:9). Platelet-poor plasma (PRP) was obtained by 15-20 minute of centrifugation at 3000 *g*. PT and aPTT (Tulip Diagnostic India) test was done a previously described [Lewis SM method]. Plasma was transferred to Eppendorf tube without delay and stored at −80°C for further use. The study was approved by institutional ethic committee of C.S.M. Medical University, Lucknow, UP, India. Written informed consent was obtained from each patient. Protein C and Protein S estimation was done by Hemostar Coagulometer (Tulip Diagnostic India).

Statistical analysis

The results are expressed as mean ± standard deviation (SD) and percentages with 95% confidence interval (CI) and range. For continuous data, the independent-sample *t* test was used to compare two means. A $P < 0.05$ was considered statistically significant.

RESULTS

The average PT, aPTT, Protein C, and Protein S in patients of gastrointestinal carcinoma was less as compared with control. The mean level of Protein C and Protein S ranged from 55% to 90% and 48% to 95%, respectively [Table 1]. Out of the 80 patients, 7 were Protein C deficient and 5 were Protein S deficient. However, three were both Protein C and S deficient [Table 2]. The Protein C level was significantly lower ($P < 0.0001$) in Protein C deficient patients with metastasis compared with patients without metastasis. Similarly, the Protein S level was significantly lower ($P < 0.0001$) in protein S deficient patients with metastasis as compared with patients without metastasis. The Protein C and S levels were also lower in those who were deficient with metastasis [Table 2]. Stages of gastrointestinal carcinoma with and without metastasis in Protein C are presented in [Table 3] and Protein S are presented in Table 4.

DISCUSSION

Cancer cells can activate coagulation directly through an interaction with platelets and/or clotting and fibrinolytic systems to generate thrombin. Clotting activation may be considered as a special type of inflammatory reaction to stimuli such as vessel wall damage, or intravascular cell aggregation or entry in blood of abnormal cells such as tumor cells. The balance between the coagulation and the fibrinolytic system can easily shift to a prothrombotic state in cancer, through an excess of tissue factor (TF), other procoagulant

Table 1: Protein and prothrombin levels in patients of gastrointestinal carcinoma as compared with control

Variables	Control Mean±SD	Patients Mean±SD
Prothrombin time (seconds)	14.05±1.89	13.09±1.45
Activated partial prothrombin time (seconds)	24.25±1.38	22.89±1.97
Protein S (%)	80.25±9.75	76.3±9.6
Protein C (%)	82.65±9.35	81.0±7.4

Table 2: Comparison of protein levels in protein deficient with metastasis without metastasis

Variables (%)	Normal without metastasis	Protein deficient with metastasis	*P* value
Protein S	78.0±8.2 (*n*=75)	58.9±4.9 (*n*=5)	<0.0001*
Protein C	82.0±6.1 (*n*=73)	65.0±6.1 (*n*=7)	<0.0001*
Both			
Protein S	76.9±9.3(*n*=77)	61.3±1.2 (*n*=3)	<0.005*
Protein C	81.7±6.3 (*n*=77)	61.7±5.8 (*n*=3)	<0.0001*

*Significant (unpaired *t*-test)

Table 3: Level of Protein C and stages of GI carcinoma with and without metastasis

Level of Protein C (%)	No. of patients *n*=80	Stage of gastrointestinal carcinoma with and without metastasis			
		1	2	3	4
0-10					
11-20					
21-30					
31-40					
41-50	7		3		4 with metastasis
51-60	3	1			2 with metastasis
61-70					
71-80	12	12			Without metastasis
81-90	25	12	13 without metastasis		
91-100	30	15	15 without metastasis		

Table 4: Level of Protein S and stages of GI carcinoma with and without metastasis

Level of protein S (%)	No. of patients *n*=80	Stage of gastrointestinal carcinoma with and without metastasis			
		1	2	3	4
0-10					
11-20					
21-30					
31-40	2		1		1 with metastasis
41-50					
51-60	3		2		1 with metastasis
61-70					
71-80	10	8	2 without metastasis		
81-90	20	18	2 without metastasis		
91-100	20	19	1 without metastasis		
101-110	25	16	9 without metastasis		

proteins or of Plasminogen activator inhibitor (PAI-1) or through deficiencies in inhibitory molecules (antithrombin, Protein C, Protein S) or in fibrinolytic principles (tissue Plasminogen activator, t-PA). Unfortunately, the true incidence of venous thrombo-embolism associated with various tumor types remains unknown for the majority of cancers because the appropriate cohort studies have not been conducted. On the contrary, the most common tumor types found in patients with venous thrombo-embolism are cancers of the lung, colon, breast, and prostate. This reflects the high prevalence of these cancers in the general population. In the present study, the levels of protein inhibitors (Protein C and Protein S) and PT as well as aPTT were measured in all the patients with gastrointestinal carcinoma. The interaction between cancer cells and components of hemostatic system is complex. Cancer patients have a tendency to experience hypercoagulation, which is exerts by chemotherapy and surgery. The interaction mechanism between malignancy and coagulation system include some general response of the host to tumor cells and specific interaction of tumor cells with the blood elements (mainly platelets and leucocytes) and with hemostatic system (including fibrinolysis and clotting component).[7]

Sengul, *et al*. 2000, reported that the coagulation parameters assessed Protein C, Protein S, Antithrombin III activity, fibrinogen level, Prothrombin time (PT), activated Partial Thromboplastin Time (aPTT). They found no differences among the three groups (vascular attacks, without vascular attacks and healthy controls) with respect to Protein C, Protein S, fibrinogen level, Prothrombin time (PT), activated Partial Thromboplastin Time (aPTT).[8]

Ozyılkan, *et al*. reported that Protein C and S levels have been found to be significantly decreased in breast cancer and concluded that the breast cancer patients are under the risk of thromboembolism even without taking any form of treatment like surgery, chemotherapy, or hormonotherapy.[9] In our study, Protein C and Protein S slightly decreased in patients as compared with healthy individual. Ellis, *et al*. reported decreased protein C activity in colon cancer and this decrease had been found to be interrelated with deep vein thrombosis.[10] In a study by Gouin-Thibault and Samama reported that protein C and S levels have also been found to be decreased for breast cancer cases.[11] In our study, the average PT in patients of gastrointestinal carcinoma was less than from the normal value of 14 seconds. Similarly, the average activated partial PT was also less than from the normal value of 24 seconds. The deficiency of coagulation factors, platelet dysfunction, thrombocytopenia, dysfibrinogenemia and increased fibrinolysis in patients of liver disease. Conversely, decrease in antithrombin and other natural anticoagulant deficiency increases the risk of thrombosis.[12]

CONCLUSION

Our study infers that APC resistance in gastrointestinal carcinoma with metastasis may contribute thrombotic

episodes in these patients. Cancer patients including GI malignancy are at increased risk for the development of thrombotic events that contribute significantly to the morbidity and mortality of malignancy in these patients.

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