

Thrombogenesis in Patients with Breast Cancer Treated with Adjuvant CMF Protocol

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ABSTRACT

This study was planned to estimate prothrombin time (PT), partial thromboplastin time (PTT), protein C and protein S before and after 6 cycles of adjuvant CMF regimen in patients with breast cancer to determine the effect of this regimen on these coagulation parameters (mainly protein C and protein S) and its role in the hypercoagulability state among these patients. Forty patients with operable breast cancer were included in the study and were subjected to initial clinical examination, intensive laboratory investigations and routine radiological studies to exclude patients with metastatic breast cancer and those with increased risk for thromboembolism. All patients were treated with adjuvant CMF regimen for 6 cycles every 21 days, two blood samples were obtained one before starting the adjuvant CMF and another one after the completion of 6 cycles of this regimen to estimate prothrombin time (Modified Quick technique) [2], partial thromboplastin time (Modified Proctor and Rappaport technique) [13], protein C and protein S (by coagulometric assay, using fibrinometer) [18,19]. We found that the mean of the four coagulation parameters were statistically significantly decreased after CMF chemotherapy ($p = 0.017, 0.001, 0.001, 0.001$ respectively). 12 patients out of 40 showed a significant decrease below normal range in prothrombin time and protein C after adjuvant CMF ($p = 0.01$ & 0.001 respectively). Also, 16 patients out of 40 showed significant decrease in protein S below normal range ($p = 0.001$), but as regard PTT, no patient showed decrease below normal range after this regimen. This decrease in the natural anticoagulants protein C and protein S might indicate that there is increased liability for thromboembolism after adjuvant CMF, although non of the patients manifested any thromboembolic complications. So, we can conclude that, patients with decrease in protein C and protein S after chemotherapy should be monitored closely to prevent thromboembolic complications, patients with heterozygous protein C deficiency or factor V leiden must be excluded for adjuvant chemotherapy by CMF and treated by another protocol and lastly large scale of patients and more extensive coagulation studies before, during and after chemotherapy may clarify the problem.

Key Words: Thrombogenesis - Breast cancer - CMF.

INTRODUCTION

It has been suggested that there is an increased incidence of thrombosis in cancer patients treated with chemotherapy [1]. Since this may occur during adjuvant chemotherapy when there is no clinical evidence of residual disease, the possibility of chemotherapy induced venous thrombosis is high [6]. Although, a direct causal relation between specific chemotherapeutic agents and thromboembolism is difficult to establish because of the many concurrent risk factors that are potentially involved, some such associations are becoming increasingly suspected and certain clinical patterns are emerging [7].

Goodnough, et al. [6] demonstrated that thrombosis was noted in 18% of patients treated with cyclophosphamide, methotrexate, 5-Fluorouracil, vincristine and prednisone (CMF-VP) for stage IV breast cancer.

Weiss et al. [19] also demonstrated that thrombosis occurred in (5-7%) of patients with stage II breast cancer treated with various regimens such as: cyclophosphamide, methotrexate, 5-fluorouracil, vincristine and prednisone (CMF-VP) or cyclophosphamide, methotrexate; 5-fluorouracil and BCG (CMF-BCG) Or CMF alone.

CMF regimen used as adjuvant therapy for carcinoma of breast may contribute to a hypercoagulable state through decreased levels of protein C and S, leading to enhanced thrombogenesis [16].

Several studies have reported an increased risk of thromboembolism in patients receiving

combination chemotherapy for breast cancer [8].

Also, more thromboembolic events had been observed with the addition of CMF chemotherapy to tamoxifen as adjuvant therapy, combination therapy was associated with more thromboembolic events (14% vs 3%) and most of the events occurred while the patients were receiving chemotherapy [12].

PATIENTS AND METHODS

Patients:

This study had been carried out in Medical Oncology and Hematology unit, Zagazig University Hospitals during the period between May, 1997 and February 1998. Forty patients with operable breast cancer underwent modified radical mastectomy at the surgical department and referred to our unit for adjuvant chemotherapy. Their ages ranged from 35-55 years (Mean 36.5 years), 34 patients with infiltrative duct carcinoma and 6 patients with lobular carcinoma. The grade of the tumor was grade I in 12 patients and grade II in 28 patients and most patients were with negative axillary lymph nodes. They had no other risk factors for thromboembolism as diabetes, hypertension, smoking, obesity.

Methods:

All patients were subjected to the following: thorough history and physical examination, routine laboratory investigations including: CBC, fasting blood sugar, blood urea nitrogen, serum creatinine and liver function tests. Radiological investigations including: chest X-ray, abdominal ultrasonography, C.T. scan of the chest, abdomen and bone scan were done when indicated to exclude metastasis.

Estimation of coagulation parameters included:

- 1- Prothrombin time (PT) [2]. Normal (11-13 seconds).
- 2- Partial thromboplastin time (PTT) [13] normal (20-40 seconds).
- 3- Estimation of protein C, coagulometric assay, using fibrintimer [18].
- 4- Estimation of protein S, coagulometric assay, using fibrintimer [19].

Estimation of protein C:

Principle:

Protein C in the patients sample is activated

by a specific snake venom activator. Activated protein C inhibits factor V and factor VIII contained in the added protein C deficient plasma. This inhibition reaction prolongs the subsequent PTT test. The prolongation of the PTT is thus a measure of the protein C content of the patients sample. Serial dilutions of a standard plasma permit a standard curve to be established from which the protein C content of the patient samples can be read in percent of the normal.

Normal range of protein C: 70-140%.

Estimation of protein S:

Principle:

Protein C_a proteolytically cleaves factor Va which is generated during the activation of the coagulation cascade by RVV (venom of *Vipera Russellii*). In this reaction protein S acts as a co-factor which powerfully accelerates the reaction. As a result, the coagulation time increases proportionally to the activity of protein S in the sample. The addition of protein S deficient plasma ensures that the test mixture has a sufficient supply of fibrinogen, factor V and the other necessary coagulation factors. Coagulation is triggered at the level of factor X by factor X activator generated by RVV. Factor Xa forms thrombin from prothrombin under the action of remaining factor Va. The resulting thrombin finally converts fibrinogen to fibrin clot. The coagulation time can be detected mechanically using the fibrintimer. The results were obtained using a reference curve prepared by serially diluting a freshly pooled plasma or a standard plasma e.g. standard human plasma, with protein S deficient plasma.

Normal range of protein S: 70-124%.

Two blood samples were taken, one sample before starting adjuvant CMF regimen, the second one after completion of 6 cycles of adjuvant CMF regimen to estimate these coagulation parameters. Dosage of adjuvant CMF used in the study was cyclophosphamide 600 mg/I.V. day I, 5-Fluorouracil 600 mg/m² I.V. day I and Methotrexate 40 mg/m² I.V. day I to be repeated every 21 days.

Statistical analysis:

Data were entered and analyzed using Epi-info version 6.02 software [5].

Paired t test: For comparison before and after treatment of the same group.

McNemar Chi square test: For comparison of two proportions-paired observation.

A significant value was considered at $p \leq 0.05$.

RESULTS

Table (1) showed that the mean of the 4 coagulation parameters (PT, PTT, protein C and protein S) were significantly decreased after ad-

juvant CMF regimen ($p = 0.017, 0.001, 0.001$ and 0.001 respectively).

Table (2) showed the number and percentage of patients who showed a significant decrease (below the normal range) in PT, protein C and protein S after adjuvant CMF regimen, 12 patients out of 40 showed a significant decrease in PT ($p = 0.01$) and protein C ($p = 0.001$) and 16 patients out of 40 showed a significant decrease in protein S ($p = 0.001$).

No patients showed decrease of PTT below normal range after CMF regimen.

Table (1): The mean values of PT, PTT, protein C and protein S before and after adjuvant CMF.

Test	Before chemotherapy $\bar{X} \pm SD$	After chemotherapy $\bar{X} \pm SD$	Paired t	p
Prothrombin time (sec)	12.35±0.74	11.49±0.80	2.449	0.017
Partial thromboplastin time (sec)	30.01±3.50	27.09±3.97	4.58	0.001
Protein "C"%	110.55±15.50	82.80±25.60	4.08	0.001
Protein "S"%	98.00±17.16	78.80±18.40	4.58	0.001

Table (2): Number and percentage of patients who showed a decrease in PT, protein C and protein S below the normal range after adjuvant CMF.

Prothrombin time	Before chemotherapy		After chemotherapy		McNemar's X^2	p -value
	No.	%	No.	%		
< 11 sec	4	10	12	30	5.25	0.01
> 11 sec	36	90	28	70		
Total	40	100	40	100		
<i>Protein C:</i>						
< 70%	0	0	12	30	11.90	0.001
> 70%	40	100	28	70		
Total	40	100	40	100		
<i>Protein S:</i>						
< 70%	2	5	16	40	13.92	0.001
> 70%	38	95	24	60		
Total	40	100	40	100		

DISCUSSION

Many patients with cancer are in a hypercoagulable state. Thrombotic episodes can present in several ways, including migratory superficial thrombophlebitis (Trousseau's syndrome), deep venous thrombosis and other sites of venous thrombosis, non bacterial thrombotic endocarditis with arterial thromboembolism, disseminated intravascular coagulation or thrombotic microangiopathy [1].

In cancer and leukemia group B (CALGB) study reported by Weiss et al. [20] a 5% incidence of thrombosis occurred at median of 3-5 months after mastectomy while the patients were receiving adjuvant chemotherapy. No patient developed thrombosis after chemotherapy was completed and there were nearly equal distribution of thromboembolic events among the three treatment groups: CMF plus vincristine and prednisone (CMFVP), CMF alone and CMF plus methanol extraction residue (MER)

of bacillus Calmette-Guerine immunotherapy.

Tamoxifen, given alone, in the adjuvant setting for breast cancer, seemingly was associated with little, if any increase in thromboembolic events [15]. However, activated protein C resistance due to factor V Leiden increases the risk of thrombosis in patients who receive tamoxifen therapy [21]. And there is a significant additional procoagulant effect when tamoxifen is added to chemotherapy [11].

The etiology of the increased incidence of thromboembolic events in breast cancer patients receiving chemotherapy is not known. Canobbio et al. [3] studied 49 patients receiving adjuvant chemotherapy for stage II breast cancer patients who were evaluated before starting chemotherapy and before each cycle. A shortening of the thrombin time and partial thromboplastin time were observed during chemotherapy.

In the present study, the prothrombin time (PT) and partial thromboplastin (PTT) were significantly decreased after adjuvant CMF chemotherapy. Nearly similar results were observed by Canobbio et al. and Saphner et al. [3,16].

Blood fluidity is maintained through a balance between the procogulant activity and the coagulation inhibitors activity. Protein C system consists of two vitamin K-dependent proteins: protein C, a natural anticoagulant which acts by inactivating active factor V and VIII and protein S which potentiates the anticoagulant activity of activated protein C. Thrombomodulin is an endothelial cell lipoprotein which acts as a cofactor in the thrombin activation of protein C [14].

In the present study the specific coagulation parameters protein C and protein S, were also significantly decreased after CMF regimen. Twelve patients out of 40 showed a significant decrease in PT and protein C below normal range and 16 out of 40 patients showed similar significant decrease in protein S (below normal range) after this regimen.

Marder [10] reported that this may fall into the range described in individuals with congenital heterozygous protein C or protein S deficiency. We noticed that this decrease was observed after adjuvant CMF and not before, reflecting the effect of this adjuvant chemotherapy for breast cancer on these specific coagula-

tion parameters. However, the mechanism for such depression of both protein C and protein S in our study after adjuvant CMF is unclear.

Our results concerning protein C and protein S, go hand in hand with those of Rogers et al. and Saphner et al. [14,16], who found that CMF chemotherapy depletes protein C and protein S leading to enhanced thrombogenesis.

These coagulation alterations found in our patients after adjuvant CMF regimen favour thromboembolic disease as there is decrease in PT, protein C and protein S.

Seiffer et al. [17] raised the possibility that antineoplastic drugs may impair either vitamin K absorption or metabolism. The postulated impairment of vitamin K metabolism caused by chemotherapy would explain the associated decrease in plasma levels of protein C and protein S noted in our study.

A second possible mechanism would involve the known inhibition of DNA and RNA synthesis by CMF chemotherapy, leading to decrease in protein synthesis in the liver and a decrease in plasma levels of protein C and protein S [1].

Still another explanation would be the initiation of intravascular coagulation by chemotherapy in patients with breast cancer. DIC is associated with decreased plasma levels of protein C [9]. So, DIC could explain the decrease in protein C and S.

In our study, none of the patients developed a clinically apparent thrombotic events. Similar results were observed by Heally et al. [7], but on the other hand Weiss et al. [20] found 5% incidence of thrombosis occurred at a median of 3-5 months after mastectomy while the patients were receiving adjuvant CMF chemotherapy. This is not surprising in view of the relatively smaller number of our patients studied over a limited time.

In congenital heterozygous deficiency of protein C, only 50% of patients develop evidence of thrombosis by 30 years of age [10]. Since CMF chemotherapy is associated with significant decrease in protein C and protein S [16], it is possible that these patients with low plasma protein C and S before treatment are more at risk for developing thrombotic events with CMF chemotherapy.

In conclusion, patients with decrease in protein C and protein S after chemotherapy should be monitored closely to prevent thromboembolic complications. Patients with heterozygous protein C deficiency or factor V leiden must be excluded for adjuvant chemotherapy by CMF and treated by other protocol. Lastly evaluation of larger patients samples including patients studied over sequential cycles of chemotherapy and patients developing clinically evident thromboembolic events, would explain or help to define the role of the observed decrease in plasma protein C and protein S in the aetiology of the enhanced thrombotic state existing in women receiving chemotherapy for breast cancer.

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