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PROTEIN C, PROTEIN S AND ANTITHROMBIN III LEVELS IN BREAST CANCER

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BACKGROUND: The increased risk for thrombosis is also valid for breast cancer. Protein C, protein S and antithrombin III levels that are all representative of inhibitory mechanism of coagulation were evaluated for a group of women with breast cancer.

METHODS: Twenty four patients with the histopathological diagnosis of invasive ductal carcinoma of the breast, 10 patients with benign breast disease and 10 control patients without any history of breast disease have been studied.

RESULTS: Antithrombin III levels for breast cancer group, benign breast disease group and control group were 110.03 ±17.41%, 97.16±8.64%, 93.10±12.24% respectively. At statistical analysis, there was a significant difference between breast malignancy and benign breast disease group and control group (p=0.034, p=0.009 respectively). Protein C levels for breast cancer group, benign breast disease group and control group were 86.25±36.23%, 114.90±7.47% and 119.20±13.30% respectively. At statistical analysis, there was a significant difference between breast malignancy and benign breast disease group and control group (p=0.023, p=0.015 respectively). Protein S levels for breast cancer group, benign breast disease group and control group were 59.12±23.25%, 99.40±7.64% and 98.90±17.71% respectively. At statistical analysis, there was a significant difference between breast malignancy and benign breast disease group and control group (p=0.0001, p=0.0001 respectively).

CONCLUSIONS: As it is the case for many cancers, natural anticoagulative pathways are also altered for breast cancer. Therefore, every precautions to decrease thromboembolism should also be taken for breast cancer patients.

MEME KANSERINDE PROTEİN C, PROTEİN S VE ANTİTROMBİN III SEVİYELERİ

ÖZET

Artmış tromboz riski meme kanseri için de geçerlidir. Bu çalışmada koagulasyonun inhibitör mekanizmalarını temsil eden Protein C, protein S ve antitrombin III seviyeleri, bir grup meme kanserli kadında çalışılmıştır.

MATERYAL VE METOD: Histopatolojik olarak memenin invaziv duktal karsinomu tanısı konmuş 24 hasta, benign meme hastalığı olan 10 hasta ve meme hastalığı hikayesi olmayan 10 kontrol hastası çalışmaya alınmıştır.

BULGULAR: Meme kanseri, benign meme hastalığı ve kontrol gruplarının antitrombin III seviyeleri sırasıyla %110.03±17.4, %97.16±8.64, %93.10±12.2 olarak bulunmuştur. İstatiksel değerlendirmede meme kanseri ile benign meme hastalıkları ve kontrol grupları arasında anlamlı fark saptanmıştır (sırasıyla p=0.034, p=0.009). Meme kanseri, benign meme hastalığı ve kontrol gruplarının protein C seviyeleri sırasıyla %86.25±36.23, %114.90±7.47 ve %119.20±13.30 olarak bulunmuştur. İstatiksel değerlendirmede meme kanseri ile benign meme hastalıkları ve kontrol grupları arasında anlamlı fark saptanmıştır (sırasıyla p=0.023, p=0.015). Meme kanseri, benign meme hastalığı ve kontrol gruplarının protein S seviyeleri sırasıyla %59.12±23.25, %99.40±7.64 ve %98.90±17.71 olarak bulunmuştur. İstatiksel değerlendirmede meme kanseri ile benign meme hastalıkları ve kontrol grupları arasında anlamlı fark saptanmıştır (sırasıyla p=0.0001, p=0.0001).

SONUÇ: Birçok kanserde olduğu gibi, meme kanserinde de doğal antikoagulan mekanizmalar değişmiştir. Bu nedenle meme kanseri hastalarında da tromboembolizmi azaltmak için her türlü önlem alınmalıdır.

Breast cancer is the leading cause of cancer related death among the women aged between 40 and 44 (**1**). It's the most frequent organ specific cancer and affect fairly wide range of population (**2**). Breast cancer frequently metastases to bone, lung, liver and central nervous system and death is due to these distant metastasis in majority of the cases. There are some other reasons for additional morbidity and mortality in breast cancer. These are a group of clinical findings called paraneoplastic syndroms. Besides the organ dysfunction caused by primary and metastatic tumors, these paraneoplastic pathologies brings additional problems to the patients and clinicians. Paraneoplastic syndromes are mediated by the cytokines produced from the tumor cells or immunological and inflammatory response of the host to the tumor cells. Thromboembolic events are also among the most frequent paraneoplastic diseases seen in the breast cancer.

The most frequent reason for cancer related thromboembolic events in females is the breast cancer (**3**). Additionally, current treatment modalities like surgery, chemotherapy and hormonotherapy increase the risk of thromboembolic events (**3**).

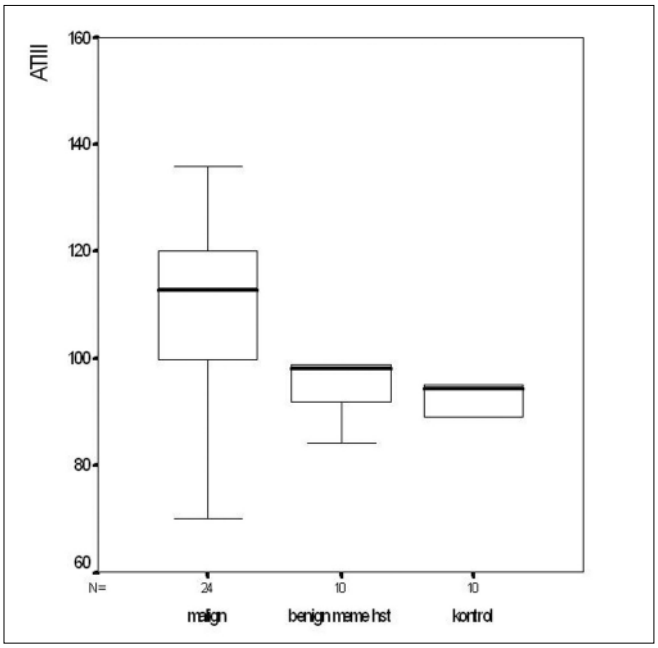

Figure 1. Antithrombin III levels (for breast cancer, benign breast diseases and control patients)

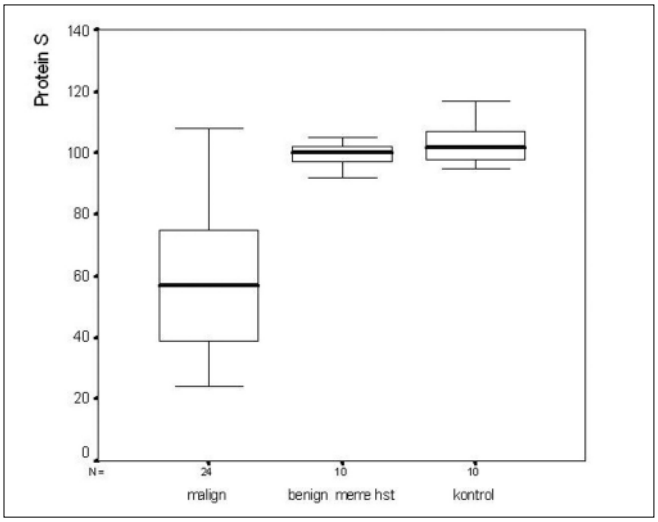

Figure 3. Protein S activities (for breast cancer, benign breast diseases and control patients)

The pathogenic mechanism of the thromboembolism seen in the breast cancer is tried to be explained by a group of complex mechanisms. In this study, protein C, protein S and antithrombin III levels that are all inhibitory system indicators for coagulation are examined in a group of breast cancer patients and their coagulative affinity with different stages of breast cancer and without any clinical sign of thromboembolism is examined.

Protein S, a vitamin K-dependant plasma protein, stimulates the proteolytic inactivation of Factor Va and Factor VIIIa. It occurs in the plasma both as a free, physicologically active form, and as well as in bound, physicologically inactive form. Diminished pro-

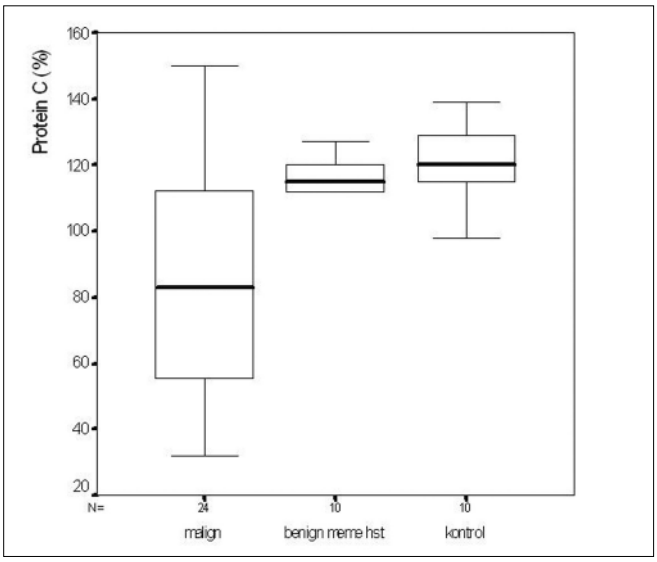

Figure 2. Protein C activities (for breast cancer, benign breast diseases and control patients)

tein S activity increases the thromboembolic risk. The causes of diminished protein S activities are hereditary protein S deficiency, hepatic disorders, oral anticoagulation, treatment with L-asparaginase, pregnancy, oral contraceptives, estrogen therapy and elevated plasma levels of C4bBP as an acute phase reaction.

Protein S acts as a cofactor in the proteolytic cleavage of FVa by activated protein C. As a result coagulation time increases proportionally to the activity of protein S in the sample. Protein C is again a vitamin K-dependant coagulation inhibitor, which regulates the activity of factor V and VIII. The induction of oral anticoagulant therapy and vitamin K deficiency may lead to very low levels of protein C activity. Factor V Leiden mutation, highly elevated activities of factor V and Lupus anticoagulants are reported to interfere with the measurements of protein C.

Material and Methods

In this study, 34 patients complaining a palpable mass in their breast, have been evaluated between January 2002 and December 2003. Ten women at the same age group without any history of malignancy and breast disease has been studied as control group. None of the patients had been operated with this complaint or other reasons before admission for 15 days. None of them had taken oral anticoagulants, aspirin, nonsteroidal anti-inflamatory drugs, oral contraseptives and hormone replacement therapy in the last one month. In their history, there was no deep vein thrombosis, myocardial infarction, cerebral ischemia and coagulopathy. Venous blood samples has been taken from superficial arm veins before biopsies. The samples has been put into citrated laboratory tubes, centrifuged immediately at 3000 rpm for 10 minutes and kept at -20°C. No platelets and leucocytes were witdrawn when removing the plasma.

Protein S Ac Kit was used with Dade Behring analyzers (measuring range: 10 to 130% of normal). Reference intervals for women without taking oral contraseptives were 50% to 118% (median: 90%). The addition of deficient plasma ensures that the test mixture has a sufficient supply of necessary coagulation factors. The results are obtained using a reference curve prepared by serially diluting standart human plasma with protein S deficient plasma and functionally active protein S is detected.

Protein C Reagent used with Dade Behring analyzers actually detects essential functions of activated protein C (inhibition of factor V and VIII). This inhibition results with prolongation of APTT testing. The results are reported in percent of normal protein C activity. The expected values range from 70 to 140% of normal (measuring range: 10 to 140% of normal).

Statistical analysis has been performed by using SPSS 11.0 for Windows. The results has been expressed as mean ± standart error. The distribution of groups has been examined with Kolmogorov-Smirnov test. Comparisons between the groups with the normal distribution and variance has been made with independent t test. On the other hand, the comparisons between the groups with abnormal distribution and/or variance has been made with Kruskal Wallis test. P values less than 0.05 has been accepted as significant.

Results

Fourtyfour women have been accepted for study. There were 24 women with breast cancer in the first group. Ten women with the benign breast diseases formed the second group and there were 10 patients in the control group. All the patients in the first group have the histological diagnosis of invasive ductal carcinoma and 3 patients were in stage I, 11 patients were in stage II, 7 patients in stage III and finally 3 patients were in stage IV. There were 5 patients with ductal ectasia, 2 patients with ductal hyperplasia and 3 patients with fibroadenomas in the benign breast disease group. The control group has been consisted of 4 patients with cholelithiasis, 1 patient with inguinal hernia, 1 patient with pilonidal sinus and 4 patients with euthyroid multinodular goitre.

Average ages of the patients with breast carcinoma, benign breast disease and control group were 50.33±2.79 (range:30-79), 50.40±4.60 (range:36-85) and 51.00±3.90 (range:35-78) respectively.

Antithrombin III levels for breast cancer group, benign breast disease group and control group were 110.03 ±17.41% (range: 70-136), 97.16±8.64 % (range: 84-112), 93.10±12.24% (range: 72-112) respectively (Figure 1). At statistical analysis, there was a significant difference between malign breast disease group and benign breast disease group and control group (p=0.034, p=0.009).

Protein C levels for breast cancer group, benign breast disease group and control group were 86.25±36.23 % (range:32-150), 114.90±7.47 % (range:98-127) and 119.20±13.30 % (range:98-132) respectively (Figure 2). At statistical analysis, there was a significant difference between malign breast disease group and benign breast disease group and control group (p=0.023, p=0.015).

Protein S levels for breast cancer group, benign breast disease group and control group were 59.12±23.25 % (range:24-108), 99.40±7.64 % (range:84-114) and 98.90±17.71 % (range:52-117) respectively (Figure 3). At statistical analysis, there was a significant difference between malign breast disease group and benign breast disease group and control group (p=0.0001, p=0.0001).

Discussion

Three factors responsible for thrombosis first described by Rudolf Wirchow are stasis, endothelial damage and hypercoagulability. Stasis is the one of most important factors for lower extremity deep vein thrombosis (DVT). Upper parts of the valvuler system of the deep veins harbours a suitable environment for stasis. Long durated immobilisation increases the probability of stasis. Besides; shock, infection, trauma, congestive heart failure, advanced age, obesity, pregnancy and intraabdominal masses may also cause stasis. The most frequent causes of venous thrombosis due to endothelial damage are venous catheters and surgical manipulations. Hereditary conditions (like antithrombin III deficiency, protein C and protein S deficiency, factor V Leiden mutation), plasminogen and thrombocyte reactivation abnormalities, factor XII deficiency, acquired conditions (like malnutrition, nephrotic syndrome, cancer, pregnancy, oral contraceptives), acute phase reactions (trauma, surgery), antiphospholipid antibodies, hyperviscosity syndromes and heparin related abnormal thrombocyte aggregation are other causes of hypercoagulability.

The relationship between cancer and thrombosis is first described by Armand Trousseau in 1865. Afterwards, deep vein thrombosis, pulmonary embolism, migrating thrombophlebitis, arterial thrombosis, nonbacterial thrombotic endocarditis, thrombotic microangiopathy and disseminated intravascular coagulation (DIC) like thromboembolic events are found to be related to cancer in many clinical and authopsy studies.

It has been stated in some studies that deep vein thrombosis might be the first clinical evidence of a malignancy. Aderka et al. has detected malignancy in 34% of their healty adult population with idiopathic deep vein thrombosis within 24 months of diagnosis. In this study, earliest cancer diagnosis was within the first year and their histopathologic types were breast and gynecologic cancers (**4**). In another study by Sorensen; the rate of breast cancer detected within the first year of deep vein thrombosis was increasing from 4.3% to 7.9% and pancreatic carcinoma rate was decreasing from 8% to 3.6% with one year follow-up (**5**). It has been claimed that bilateral recurrent deep vein thrombosis, resistant to treatment, might be the evidence of a hidden cancer (**3**). In many retrospective studies the incidence of cancer in the population with and without DVT has been investigated . By evaluating

all these studies, the risk of malignant disease has been found to be doubled in the group of patients with DVT (**6**). More recently, higher rates of malignancy has been reported for patients with DVT in two retrospective studies (**7,8**). Sorensen et al. had reported increased risk of malignancy diagnosed after idiopathic DVT as 3 times higher for first 6 months, 2.2 times higher for the first year and 1.1 times higher for the following years (**7**). Baron et al. has reported increased risk of malignancy even after 10 years (**8**). The risk of cancer after bilateral and recurrent DVT was found to be higher (**9**). The evaluation of patients with idiopathic DVT for the evidence of a hidden malignancy and thus the detection of a cancer in early stages has been discussed. But systemic physical examination, occult blood testing in stool, gynecological examination, mammography, chest X-ray and urological examination for males are accepted to be sufficient by many authors. In case of strong cancer suspicion, computed tomography, endoscopy and tumor markers may be used (**3**). In a study by Montreal et al. 31 patients with idiopathic DVT have been evaluated with abdominal ultrasonography, upper gastrointetinal endoscopy, blood tests and computed tomography and 7 cases of early staged cancers have been diagnosed (**10**). In a study by Piccioli, the cancer related mortality rates were found to be similar for the patients with or without diagnostic intervention for the diagnosis of occult cancer (**11**). In the study of Sorensen mentioned earlier, the survival of cancer patients with and without the history of DVT has been compared and the prognosis of the patients with the history of DVT has been found to be worse (**5**).

The pathogenesis of prothrombosis in cancer is complex. The integration of activated coagulative and fibrinolytic systems, vascular endothelial damage and activation of monocytes and thrombocytes by citokines are the main topics of discussion. The fundamental change is the activation of inflammatory process. Tumor cells activate coagulation cascade with direct and indirect mechanisms. Direct mechanisms work on tissue factor and activation of cancer procoagulants. Tissue factor is a membrane protein and its occurrance in lung, prostate, breast and renal cancers, leukemias and lymphomas has been proven. Tissue factor and cell differentiation are interrelated (12,13). Tissue factor activates factor VII and forms activated factor IX and X complex and increases thrombin. Thrombin activates factor V, VIII and IX and this activation increases the protein C activity indirectly. Direct or indirect activation of coagulation cascade works for neoplastic dissemination and metastasis. This effect is a part of angioneogenesis (14). Cancer procoagulant is a calcium dependant cystein protease and found in tumor cells of melanoma, colon, lung and breast carcinoma and fetal tissues. Another thrombogenic mechanism is the activation of monocytes, endothelial cells and lymphocytes by the tumor cells. These cells activate immune and inflammatory response with the help of cytokines. The result is the activation of complement system and formation of immune complexes. Stimulated monocytes and endothelial cells activate tissue procoagulants (15). Besides all these, complex defects may occur in natural inhibitory mechanism of coagulation that are antithrombin III, protein C and protein S. As in the case in our study and two other studies in the literature with elevated antithrombin III levels, the exact mechanism can't be explained (16,17). The increase of antithrombin III activity is not an expectation. Wojtukiewicz et al. have reported normal antithrombin III levels in patients with melanoma, breast cancer and stomach cancer (18). In a study by Honeggar et al. antithrombin levels have been found to be decreased in metastatic cancer patients and thought that, antithrombin III levels may be affected by the treatment modalities that affect coagulopathic tendency adversely (19). In a study, the high levels of antithrombin III was found to be decreased even lower than normal levels in a group of patients taking tamoxifen (20). Increased thromboembolism risk with tamoxifen is related with its intrinsic estrogenic activity and its negative effect on antithrombin III, protein C and S levels. But the laboratory results describing the exact mechanism has not been reached yet (21). The patients with the history of thromboembolism should be evaluated before starting tamoxifen therapy.

In a study by Ozyılkan et al. thrombin-antithrombin III complex levels were found to be increased significantly in breast cancer. In this study, CA 15-3 levels were also measured and significant correlation between increased CA 15-3 and thrombin-antithrombin complexes have been detected (**22**). But the measurement of thrombin-antithrombin III complex is quite expensive laboratory test. In the same study; protein C and S levels have been found to be decreased significantly in breast cancer and said that breast cancer patients are under the risk of thromboembolism even without taking any form of treatment like surgery, chemotherapy or hormonotherapy. Ellis et al had reported decreased protein C activity in colon cancer and this decrease had been found to be interrelated with deep vein thrombosis (**23**). In another study by Gouin-Thibault et al; protein C and S levels have also been found to be decreased for breast cancer cases (**24**). Our study findings are in accordance with the increased risk of thrombosis for the cancer patients.

Cancer itself prepares the ground for thromboembolic complications. Cancer treatment with surgery, chemotherapy and hormonotherapy also increases this risk. Its important to prevent thromboembolism with respect to the quality of life. Althogh the preventive measures like early mobilisation, selective use of central venous catheters may be effective, the most important preventive measure is the use of anticoagulants. We suggest standardized use of thromboembolism prophylaxis for breast cancer patients.

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