Elevated Plasma Levels of the Tissue Factor Pathway Inhibitor (TFPI) in Gynecologic Cancers

Jinekolojik Malignitelerde Doku Faktör Yolu İnhibitörün (TFPI) Artmış Düzeyleri

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ABSTRACT Objective: Malignant cells increase the expression of procoagulant substances like Tissue factor (TF). A kind of natural protease, called TF pathway inhibitor (TFPI), that hinders the connection of TF and inhibits the activation of extrinsic coagulation pathway was detected. **Material and Methods:** 59 cases which were interned to operate for gynecologic tumors were included to take part in this study. Also, 14 healthy subjects were enrolled as controls to the study. The TFPI's activities of the plasma samples which were frozen were measured by the assay kit of TFPI's activitiy (Actichrome). The differences in means for groups (malignant/ benign & control/benign) for continuous variables of TFPI's levels were tested by t-test for two independent groups, and Mann Whitney U test was used for malignant / metastatic & nonmetastatic groups for the mean of TFPI's levels. **Results:** There was a significant difference between malignant and benign and control groups plasma levels of TFPI which was found out higher in malignant group ($p< 0.0001$), which was similar between malignant and control group also. In the malign group; plasma TFPI levels of metastatic (stage 3-4, n=11) cases were higher than non-metastatic (stage 1-2, n=21) cases (0.135 U/mL and were 0.106 U/mL) ($p=0.031$). **Conclusion:** The high levels of TFPI in malignancy are questioned if it is a mechanism of defense of body cells or a kind of metastasis mechanism of malignant cells.

Key Words: Blood coagulation; tissue factor pathway inhibitor (1-161); carcinoma

ÖZET Amaç: Malign hücreler, Tissue factor (TF) gibi prokoagülan maddelerin ekspresyonunu arttırırlar. F VII'nin aktif formuna dönüşmesini ve böylece TF ile bağlanmasını engelleyen 'TF pathway inhibitor'(TFPI) olarak adlandırılan doğal bir proteaz varlığı tespit edilmiş bulunmaktadır. **Gereç ve Yöntemler:** Bu çalışmaya jinekolojik tümör nedeniyle ve opere edilen 59 olgu dahil edildi. Kontrol grubu olarak, herhangi bir sistemik ve jinekolojik problemi olmayan 14 olgu alındı. Dondurularak saklanan plazma örneklerinin TFPI aktiviteleri, Actichrome® TFPI aktivite assay kiti ile ölçüldü. Malign-benign, kontrol-benign olguların TFPI düzey ortalamalarının karşılaştırılmasında bağımsız iki grubun standart t testi, malign, metastatik-nonmetastatik grupların TFPI düzeylerinin karşılaştırılmasında Mann-Whitney U testi kullanıldı. **Bulgular:** Malign olguların TFPI düzeyleri ortalamaları, benign olguların ve kontrol grubunun TFPI düzeyleri ortalamalarından istatistiksel olarak ileri derecede anlamlı yüksek tespit edildi ($p<0.0001$). Malign olgular içinde nonmetastatik (evre 1-2) olan grubun serum TFPI düzeyleri (0.106 Ü/ml), metastatik (evre 3-4) olan grubun serum TFPI düzeylerinden (0.135 Ü/ml) düşük olup istatistiksel olarak anlamlı fark saptandı ($p=0.031$) **Sonuç:** Malign olgularda TFPI düzey artışı konak hücrelerinin bir savunma mekanizması mı yoksa tümör hücrelerinin bir yayılma mekanizması mı olduğu sorularını akla getirmektedir.

Anahtar Kelimeler: Koagülasyon; doku faktör yolu; inhibitörü (1-161); malignite (karsinoma)

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Most patients with malignant disease have evidence of increased coagulation activation. Thrombosis is a frequent complication of malignancy and the second most important cause of death in cancer patients.[1,2] For many years, these clinical observations have triggered interest in blood clotting activation in cancer patients and, more recently,

in the possibility that such activation plays a role in cancer growth, particularly in metastatic development.

Malignant cells constitutively express procoagulants such as tissue factor (TF). TF which complexes with factor VIIa (FVIIa) and activates factor X (FX) which is a cysteine protease.[3,4] Activated FX (FXa) initiates extrinsic pathway of the blood coagulation which mechanism can explain why the cancer patients have hypercoagulability.[5] A kind of natural protease, called TF pathway inhibitor (TFPI), that inhibits the binding of TF and FVI-I by blocking the activation of FX was detected.[6,7] In the most of the recent many studies, plasma levels of TFPI in malignancies of colon, breast, lung and hematology were searched and, it was shown that plasma levels of TFPI increased in solid tumors.[8,9] However, there is no tumor specific study about this subject and the aim of this study is to determine the plasma levels of TFPI in gynecologic tumors and to search the relationship between these levels and the stage of malignant diseases.

MATERIAL AND METHODS

Fifty nine cases which were interned to operate for gynecologic tumors were evaluated in this study. In these patients, definitive pathologies weren't known before operations, however adnexial pathology (n=44), uterine pathology (n=10), cervical pathology (n=4), and vulvar pathology (n=1) were present. Fourteen cases without any systemic and gynecologic problems were included as control group. Patients with using anti-coagulant and oral contraceptive or having organ insufficiency were excluded. After the operation, pathologic findings were detected.

BLOOD SAMPLES

5 cc venous blood samples were taken to tube with sodiumcytrate before the operation from gynecologic tumor (n=59) group and control group (n=14). Plasma samples were distinguished from blood samples by centrifuging at 3000 (rpm) turning for ten minutes. Plasma samples were kept at -20°C in plastic tubes until analyzing time. Frozen plasma samples were quickly dissolved at 37°C and they were made ready for measurement.

THE MEASUREMENT OF TFPI'S ACTIVITY

The TFPI's activities of the plasma samples which were frozen were measured by the assay kit of TFPI's activitiy (Actichrome) (Product no: 848, American Diagnostica Inc). The assay kit of TFPI's activity (Actichrome®) measures TFPI by cromogenic which affects by inhibiting TF/FVIIa complex in plasma.

Procedure:

- 20 ml diluted reference plasma the samples of tests and the standards of TFPI were added to microwells.

- The complex of 20 ml TF/FVIIa was added to microwells.

- Microwells were closed and incubated at 37°C about 30 minutes.

- 20 ml FX of human was added to mixture and, it was incubated at 37°C for 15 minutes.

- 20 ml EDTA, and then 20 µl SPECTROZYME® FXa were added.

Reaction immediately starts after adding SPECTROZYME® FXa. The color of solution turned to yellow while the reaction was going on. Absorbance of solution was noted at 450 nm wavelength and the changes of color were monitorized in every 5 minutes. Glacialasetic acid like 50 ml was added in every well and the reaction was stopped at 25 minutes.

STATISTICAL ANALYZES

The difference in means for two groups (malignant/ benign &control) for continuous variables of TFPI's levels was tested by t-test for two independent groups, and Mann Whitney U test was used for malignant / metastatic & nonmetastatic groups for the mean of TFPI's levels. The statistical analyzes were made using SPSS 11 (Inc, Chicago, IL) programme working with Windows.

RESULTS

With malignant and benign tumor, 32 and 27 patients and without any gynecologic disorders 14 cases were included in this study. In malignant group (n=32), there were ovarian, endometrium, cervix

TABLE 1: Distribution of the groups.

Groups	n
Benign	27
Malignant	32
Ovary	17
Endometrium	10
Cervix	4
Vulva	1
Control	14

and vulva cancers (n=17, n=10, n= 4, n=1, respectively) (Table 1).

Means of plasma levels of TFPI were 0.081 U/mL (0.040 U/mL-0.178 U/mL), 0.116 U/mL (0.091 U/mL-0.181 U/mL), and 0.074 U/mL (0.029 U/ mL-0.120 U/mL) for benign, malignant and control groups. There was a significant difference between malignant and benign groups plasma levels of TFPI which were found out higher in malignant group ($p< 0.0001$), which was similar between malignant and control groups also. No significant difference in plasma levels of TFPI between control & benign groups was observed ($p <0.05$) (Table 2).

In the malign group; plasma TFPI levels of metastatic (stage 3-4, n=11) cases were higher than non-metastatic (stage 1-2, n=21) cases (0.135 U/mL and 0.106 U/mL) (p=0.031) (Table 3).

TABLE 2: Mean values of levels of TFPI's activity in benign, malignant and control groups.

Groups	TFPI Mean (U/ml)
Benign (n = 27)	0.081 (0.040 – 0.178)
Malignant (n = 32)	0.116 (0.09 – 0.181) *
Control (n = 14)	0.074 (0.029 – 0.120) **

p value of mean of TFPI in malignant and benign group < 0.0001

p value of mean of TFPI in malignant and control group <0.0001.

TABLE 3: Mean values of levels of TFPI's activity in metastatic and nonmetastatic cases.

Cases	TFPI mean (U/ml)
Metastatic (n=11)	0.135
Nonmetastatic (n=21)	0.106*

* p<0.05.

DISCUSSION

The activation of blood coagulation in cancer patient has been found interesting for many years. Nowadays whether this activation has a role in development of cancer or not is being discussed.

The symptoms of this activation can be found many patients with malignancy. Fibrin monomers and levels of D-dimer were found out higher in patients with solid tumors and it has been accepted as a sign of releasing of TF and the activation of FX.[9] The main mechanism(s) of high levels of TFPI in cancer patients is not clear yet. However, it has been detected that in different cancer types and high levels o f TFPI is related to progression of disease.[10,11] In our study, significant differences in plasma levels of TFPI between malignant and benign /control groups were observed which was found out higher in malignant group and we had the similar results between the metastatic malignant and nonmetastatic malignant group also. These results were harmonious with literatures. Normal levels of TFPI activity according to our assay (ACTICHROME® TFPI Activity Assay) is referred as 0.7-1.2 Ü/ml. In this study the results values in benign/malignant/control were in the normal range of assay values. But results in malignant groups (mean 0.116 U/ml), were higher in benign (mean 0.081 U/ml), non-metastatic (mean 0.106 U/ml) and control groups (mean 0.074 U/ml). This conflict can be explained as; because the assay isn't routine usage it hasn't been standardized in large groups yet.

Amirkhosravi et al. detected that coagulation activity has a part in experimental metastasis development.[12] TF effectivity in metastasis is explained by occurring along with angiogenesis. The possible antimetastatic effects of TFPI must be detected by experimental studies. The study of Amirkhosravi et al. is supported by the study of Fisher et al.[12,13] TFPI passes through extracellular space and this transition has an effective role in starting the adhesion and migration of tumor cells by TF/FVIIa complex. In this point of view, TFPI can be prometastatic. TFPI can be anticoagulant and antimetastatic in blood circulation and when it is released to

extracellular space it can be proinvazive and prometastatic.[13]

The high levels of TFPI in malignancy are questioned if it is a mechanism of defense of body cells or a kind of metastasis mechanism of malignant cells. However we don't have sufficient data to find the answer of these questions and we still need more clinical and laboratuary study. There are significant difference between TFPI activity of malignant group and control-benign group. According to these results, this parameter can be used as a serum marker to evaluate and discriminate of diagnosis.

REFERENCES

1. Ricles FR, Levine MN. Venous thromboembolism in Malignancy and Malignancy in Venous Thromboembolism. Haemostasis 1998; 28:43-9.
2. Falanga A, Donati MB. Pathogenesis of thrombosis in patients with malignancy. Int J Hematol 2001;73:137-44.
3. Donati MB, Semararo N. Cancer cell procoagulants and their pharmacological modulation. Haemostasis 1984;14:422-9.
4. Valesco F, Lopez-Pedrera F, Borrell M, Fontcuberta J, Torres A. Elevated levels of tissue factor pathway inhibitor in accute non- lymphoblastic leukemia patients with disseminated intravascular coagulation. Blood Coagul Fibrinolysis 1997;8:70-2.
5. Inversen N, Lindahl AK, Abildgaard U. Elevated TFPI in malignant desease: relation to cancer type and hypercoagulation. Br J Hematol 1998; 102:889-95.
6. Broze GJ Jr. Tissue factor pathway inhibitor and the current concept of blood coagulation. Blood Coagul Fibrinolysis 1995;1:7-13.
7. Bajaj MS, Birktoft JJ, Steer SA, Bajaj SP. Sutructure and biology of tissue factor pathway inhibitor. Thromb Haemost 2001;86:959-72.
8. Panteleev MA, Zarnitsina VI, Ataullakhanov FI. Tissue factor pathway inhibitor: a possible mechanism of action. Eur J Biochem 2002; 269:2016-31.
9. Iversen N, Lindahl AK, Abildgaard U. Elevated plasma levels of the factor Xa-TFPI complex in cancer patients. Thromb Res 2002;105:33-6.
10. Lindahl AK, Sandset PM , Abildgaard U, Andersson TR, Harbitz TB. High levels of extrinsic pathway inhibitor and low levels of other coagulation inhibitors in advenced cancer. Acta Chir Scand 1989;155: 389-93.
11. Lorenzet R, Donati MB. Blood clotting activation, angiogenesis and tumor metastasis: any role for TFPI? Thromb Haemost 2002;87:928-9.
12. Amirkhosravi A, Meyer T, Chang JY, Amaya M, Siddigui F, Desai H, et al. Tissue factor pathway inhibitor reduces experimental lung metastasis of B16 melanoma. Thromb Haemost 2002;87:930-6.
13. Fischer EG, Riewald M, Huang HY, Miyagi Y, Kubota Y, Mueller BM, Ruf W. Tumor cell adhesion and migration supported by interaction of a receptor-protease complex with its inhibitor. J Clin Invest 1999;104:1213-21.