Serum Endothelial Protein-C Receptor Levels in Patients with Breast Cancer

Meme Kanserli Hastalarda Serum Endotelyal Protein-C Reseptör Düzeyleri

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ABSTRACT

Objectives: The aim of this study is to analyze the association between soluble endothelial protein C receptor (sEPCR) levels and pathology results in patients with breast mass.
Methods: Seventy three patients with breast mass were enrolled. The epidemiologic features and pathology results were recorded. Serum sEPCR levels were analyzed by ELISA.
Results: Thirty five patients had breast cancer with sEPCR level of 130.31±89.51 ng/ml and 38 had benign breast lesions with sEPCR level of 116.58±88.68 ng/ml (p>0.05). Upon the patients with breast cancer sEPCR levels were not statistically significant between the early and late stages (134.83±91.89ng/ml and 119.01±86.9 ng/ml, respectively). Also there were no difference in patients with positive and negative Estrogen or progesterone receptors (p>0.05). But sEPCR level was obviously higher in patients with negative HER-2 receptor (149.68±102.43 ng/ml, p=0.029).
Conclusions: Although there was no statistically significant difference, the patients with malign breast masses had higher levels of sEPCR. The receptor status was found to influence the serum EPCR levels but broader series of cases are required to understand the clinical importance and to determine its potential effect on targeted treatment plan.

Keywords: Benign breast mass; breast cancer; soluble endothelial protein C receptor

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ÖZET

Amaç: Bu çalışmanın amacı, memede kitle nedeniyle opere olan hastaların patoloji sonuçlarıyla serum endotelyal protein-C resptörü (sEPCR) arasındaki ilişkiyi araştırmaktır.
Yöntem: Çalışmaya 73 hasta dahil edildi. Hastaların epidemiyolojik özellikleri ve patoloji sonuçları kaydedildi. Serum EPCR düzeyleri, ELISA yöntemi ile çalışıldı.
Bulgular: Meme kanseri tanısı alan 35 hastanın sEPCR düzeyi 130,31±89,51 ng/ml iken benign meme kitlesi olan 38 hastanın sEPCR düzeyi 116,58±88,68ng/ml idi (p>0,05). Meme kanserli hastalar incelendiğinde; sEPCR düzeyinde erken evre ve geç evre arasında istatistiksel olarak anlamlı fark saptanmadı (sırasıyla; 134,83±91,89 ng/ml ve 119,01±86,9 ng/ml). Östrojen ve Progesteron reseptörü negatif veya pozitif olan hastalar arasında da anlamlı farklılık saptanmadı (p>0,05). Fakat sEPCR düzeyleri; HER-2 negatif olan hastalarda belirgin olarak yüksekti (149,68±102,43 ng/ml, p=0,029).
Sonuç: İstatistiksel olarak anlamlı olmasa da, malign meme kitlesi olan hastalarda sEPCR düzeyleri daha yüksekti. Reseptör durumunun sEPCR düzeylerini etkilediği görülse de bunun klinik önemi ve tedavi üzerine olan potansiyel etkilerini anlamak için daha geniş vaka serileri içeren çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: Benign meme kitleleri, meme kanseri, serum endotelyal protein C reseptörü

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INTRODUCTION

The primary regulator of the coagulation system is protein C (PC) pathway. Activated PC (APC) plays an important role in inactivating active factor V and VIII. The zymogenic form of PC circulates in the blood. The PC zimogen is activated when it binds to thrombin. Thrombomodulin and endothelial protein C receptor (EPCR) have an important role in the activation of PC. EPCRs are located on the endothelial surface. Also, the PC pathway has role in the inflammation, cell death and maintaining the permeability of the blood vessels. EPCRs and the protease activated receptor-1 (PAR-1) mediate APC's cytoprotective effects, antiapoptotic activity, anti-inflammatory activity and protection of endothelial barriers (1-3).

Coagulation initiating factor is known to be released by tumor cells in many cancer types thus thrombin, platelet activation and fibrin appear and this plays a role in proliferation of cancer cells. Thrombin also induces anti-coagulant protein-C activation. Endothelial protein-C receptor plays a role in cellular signal system and metastasis development initiated by APC and these results in anti-inflammatory and anti-apopitic activities directed by protease activated receptor-1 (PAR-1). As a result APC activates the signal pathways of invasion and chemotaxis by EPCR and PAR-1 (2, 4-6).

The aim of this study is to analyze the association between soluble EPCR levels and pathology results in patients with breast mass, and also the association between soluble EPCR levels and prognostic factors in patients with malign breast disease.

PATIENTS and METHODS

In this study, consecutive 73 patients presented with breast mass were enrolled. Their epidemiologic and clinical data including physical examination, radiological and pathologic findings were obtained from their hospital charts.

The patients with malign disease were staged according to the TNM staging system. Localized disease was defined as stage 1 and 2, and advanced disease was defined as stage 3 and 4.

Blood samples for sEPCR were from the patients' preoperative periods. Blood samples were stored at -80ºC. To determine for sEPCR levels an enzyme-linked immunosorbent assay (ELISA) was used (Diagnostica Stago Asserachrom sEPCR, Asnieres-France).

This study was approved by the ethics committee of Selcuk University (2014/62), and informed consent was obtained from each patient.

The Statistical Package for Social Sciences (SPSS for Windows version 13.0, Chicago, IL, USA) program was used for assessment of the results. Median values were used to analyze demographic characteristics. The parametric data are given as arithmetic means ± standard deviation (SD) and non-parametric data are given as median (minimum–maximum). Pearson chi-square test was used to compare categorical variables and comparison between groups was determined by Student's *t* test or Mann–Whitney *U* test (parametric data or non-parametric data, respectively). In the statistical evaluations, a *p* value of < 0.05 was regarded as significant.

This study was approved by the Ethical Committee of Selcuk University Medical Faculty (No: 2014/62).

RESULTS

There were 38 patients with benign breast mass and 35 patients with invasive ductal carcinoma. The age of patients with the benign breast mass ranged from 16 to 59 years with a median age 37.5 years. The age of the patients with invasive ductal carcinoma ranged from 31 to 89 years with a median age 49 years.

The sEPCR levels of the patients with benign and malign breast masses were 116.58 ± 88.68 ng/ml and 130.31 ± 89.51 ng/ml, respectively (Figure 1). There were no differences between the patients with benign and malign breast masses (p=0.51).

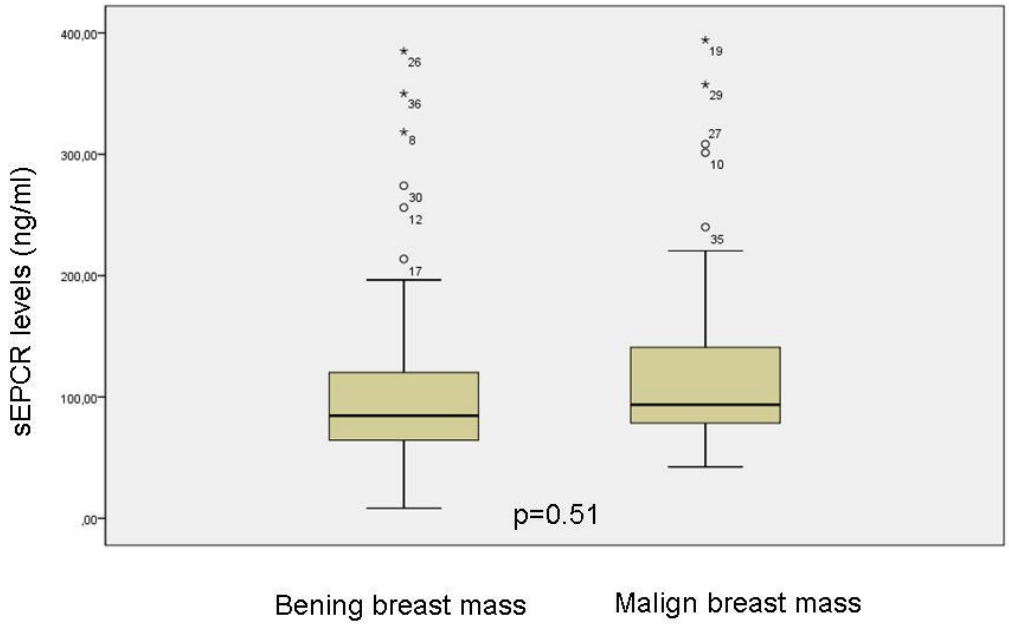

Figure 1. The patients' sEPCR levels

In the patients with malign breast masses, when the patients with malignancy were evaluated separately (Table 1), sEPCR levels of the stage I, II, III and IV were 123.63 ± 77.74 ng/ml, 140.10 ± 99.65 ng/ml, 123.46 ± 103.67 ng/ml and 108.64 ± 43.42 ng/ml, respectively. When evaluated according to the stages, the difference was not statistically significant. Also, sEPCR levels of the localized and advanced diseases were 134.83 ± 91.89 and 119.01 ± 89.9 ng/ml, respectively but the difference was not statistically significant again.

When the relationship between estrogen or progesterone receptor status and sEPCR was evaluated, there was no statistically significant difference. However, the sEPCR levels of the patients with/without HER-2 receptor were 157.27 ± 117.71 and 100.06 ± 42.00 ng/ml, respectively and this difference is statistically significant (p=0.029).

Table 1: The sEPCR levels according to the subgroups of the patients with malign breast diseases

	sEPCR levels (ng/ml)	p value
Stage		NS
I (n: 8)	123.63 ± 77.74	
II (n: 17)	140.10 ± 99.65	
III (n: 7)	123.46 ± 103.67	
IV (n: 3)	108.64 ± 43.42	
Disease status		NS
Localized disease (n: 25)	134.83 ± 91.89	
Advanced disease (n: 10)	119.01 ± 89.9	
Receptor status (n: 21)		
Estrogen		NS
Negative (n: 7)	128.04 ± 119.67	
Positive (n: 14)	126.94 ± 119.67	
Progesteron		
Negative (n: 7)	128.04 ± 119.67	NS
Positive (n: 7)	126.94 ± 75.22	
HER-2		0.029
Negative (n: 11)	157.27 ± 117.71	
Positive	100.06 ± 42	

DISCUSSION

The roles of components such as APC and PC inhibitory have an important role in hemostasis. Also, they have many roles in inflammation, proliferation and apoptosis of the cells, and also migration, invasion and metastasis development of the cancer cells. It has been reported that APC may increase the invasion and chemotaxis of breast cancer cells through EPCR and PAR-1 in breast cancer. APC has also been shown to increase the proliferation of vascular endothelial cells and angiogenesis by EPCR (4, 5).

Studies on some hemostasis components such as protein C, PAR-1, EPCR, APC are very limited in breast cancer (7-15). In Keshava and colleagues' studies (7, 8), it has been suggested that EPCR expression in breast cancer cells may limit cancer progression in advanced stage patients and that EPCR may also function as a negative regulator of cancer progression. In an important study by Schaffner et al. (9), EPCR receptor-blocking antibodies have been shown to inhibit the growth of EPCR-positive tumors in vivo. This study suggests that it may be a target molecule for cancer treatment. In another study, new relationships between common single nucleotide polymorphisms in *F5*, *F10* and *EPCR* genes and breast cancer susceptibility were shown and it was considered that new treatment strategies could be developed with these findings (10). In triple-negative breast cancer cells, EPCR expression is a characteristic of cancer stem cell-like populations. In vivo tumor growth and proliferation of EPCR positive cells have been shown to be inhibited by blockade of EPCR with antibodies (11). In mice, PC receptor positive multipotent breast stem cells were identified. It was emphasized that these cells are located in the basal layer, exhibit epithelial-to-mesenchymal transition characteristics, and have low levels of basal keratin expression. In the light of these findings, it has been suggested that a new multipotent breast stem cell population may have importance in the onset of breast cancer (12). In another study, PC receptor expression in tumor tissue of patients with invasive ductal cancer was evaluated by immunohistochemically and the clinical importance of this was investigated. It has been shown to be associated with distant metastasis. In addition, negative effects of PC receptor expression on survival rates were also shown (13). In the experimental study of Perurena et al. (14), high EPCR expression in breast tumors has been shown to be associated with poor clinical outcome. It was emphasized that silencing of EPCR impairs the development of breast tumor and metastases.

It has been shown that the silencing of SPARC/osteonectin, Cwcv and kazal-like domains proteoglycan (SPOCK1), a mediator for the effects of EPCR, has impaired breast tumor growth and inhibited the development of metastasis. In another experimental study, PC receptor expression in triple negative breast cancer was found to be high (15).

In this study, our aim was to analyze the association between soluble EPCR levels and pathology results in patients with breast mass, and analyze the association between sEPCR levels and prognostic factors in patients with malign breast disease. In our study, sEPCR was slightly higher in breast cancer patients compared to patients with benign breast mass, but the difference was not statistically significant. Similarly, there was no correlation between sEPCR and stage, estrogen receptor and progesterone receptor status of the tumor. However, sEPCR levels were significantly higher in patients with HER-2 receptor negative breast cancer.

In conclusion, EPCR blocking in triple-negative breast cancers with poor clinical outcome and in HER-2 receptor negative breast cancer patients may be a hope of a new treatment in these patients. Soluble EPCR measurements can provide quick information about the patient's EPCR status.

Conflict of interest

No conflict of interest was declared by the authors.

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