Original article

Serum levels of angiogenic factors in early breast cancer remain close to normal

Derya Duranyildiz*, Hakan Camlica, Hilal Oguz Soydinc, Duygu Derin, Vildan Yasasever

Istanbul University Oncology Institute, 34390 Istanbul, Turkey

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ABSTRACT

The study which we performed was to determine serum concentrations of angiogenic factors including VEGF, angiogenin and TGF-beta 1 in early stage breast cancer patients. These parameters were measured by ELISA in sera of 90 patients with breast cancer and 75 healthy controls. The mean serum VEGF concentration in patients compared to controls was not significantly different (0.33 ng/mL vs 0.43 ng/mL, respectively; $p = 0.156$). Likewise, the insignificant change in mean values in patients vs controls was also observed for serum TGF-beta 1 (0.19 ng/mL vs 0.19 ng/mL, respectively; $p = 0.215$) and serum angiogenin (243.24 ng/mL vs 244.5 ng/mL, respectively; $p = 0.976$). Statistically significant correlation was found only between the tests, such as VEGF and angiogenin in patients who were included in the study ($p < 0.001$). In conclusion, we couldn't find any diagnostic value between the early stage breast cancer and the three angiogenic parameters.

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Introduction

Tumor angiogenesis, which is the formation of new blood vessels, is also one of the most important biologic features that are related to tumor growth and metastasis. The tumor progression and spreading is associated with some molecules forming or triggering angiogenesis in cancer. In addition to evaluating the expression of these factors in the tumor tissue, an other indirect way to measure their activities in cancers is determining the quantity of proteins secreted into vessels. Measurement of circulating factors is a convenient and non-invasive method that is potentially applicable to every cancer patient.[1] Although elevated serum levels of these factors have been observed in the sera taken from patients various types of human cancer, little is known about its clinical significance; there is only limited information on serum levels in patients with breast cancer.[2–4]

The angiogenic phenotype depends on a net balance between positive and negative angiogenic factors released by the tumor. So far, many angiogenic factors, containing especially vascular endothelial growth factor (VEGF), which is one of the most important substances, have been shown to be produced by a variety of different tumor cells, including breast carcinoma.[1,5] The VEGF levels have been studied with different types of materials and the role has been evaluated in angiogenesis and progression of the breast cancer.[6,7]

In addition to VEGF, some of the principal angiogenic regulators, angiogenin and transforming growth factor-beta (TGF-beta) have been identified and shown to be produced by a variety of different tumor cells, as in breast cancer.[8–10]

In our last study, which was performed in all clinical stages of malignant melanoma patients, we found that serum VEGF, angiogenin and TGF-beta 1 levels were similar in all patients group with control group.[11]

In this study, we aimed at determining the serum concentrations of angiogenic factors including VEGF, angiogenin and TGF-beta 1 in early stage breast cancer patients.

Material and methods

The study group consisted of Caucasion patients consecutively presenting to our Institute of Oncology. 90 patients with pathologically verified breast carcinoma stages I and II of disease and 75 healthy controls matched in age and menapousal status were investigated. Clinical staging of patients was performed on a pathological basis according to the new American Joint Committee on Cancer (AJCC) 2001 classification system.[12] Controls were recruited from among the Institute personnel who were in excellent health at the time of the study ($n = 75$) with median age 43 (28–69) years were included as the control group.

Our study on human materials has been approved by the relevant institutional committee (Local Ethics Committee, number: 1372). Written informed consent was obtained from all patients included in the study.

Blood samples were obtained from the patients within 30 days after initial surgery; without undergoing any type of adjuvant treatment by venipuncture and clotted 2 h at room temperature.

* Corresponding author. Tel.: +90 212 4142434; fax: +90 212 2224959.
  E-mail address: dduranyildiz@yahoo.com (D. Duranyildiz).

Sera were collected following centrifugation (at 4000 rpm, 10 min) and frozen immediately at $-20\,^{\circ}$C until analysis.

Circulating angiogenin, TGF-beta 1 and VEGF levels were evaluated by solid-phase enzyme-linked immunosorbent assay (R&D Systems Inc., MN, USA) using 96-well microplates in accordance with the manufacturer's instructions. Standard curves were constructed using serial dilutions of recombinant angiogenin, TGF-beta 1 and VEGF. Each sample was tested in duplicate. The color development is stopped with the stop solution and the intensity of the color was measured at 450 nm using ELISA reader (Rayto RT-1904C Electronics Inc., China). VEGF, TGF-beta 1 and angiogenin levels were expressed as nanogram per milliliter (ng/mL).

Data analyses were performed by using SPSS software (SPSS 16, Chicago, IL, USA). The values of serum angiogenin, TGF-beta 1, VEGF, were described as mean (x), standard deviation (sd), median (m), minimum (min), and maximum (max) of values. In comparison with median values of serum levels, statistical significance was determined with the Mann–Whitney U test. Correlations were calculated with Spearman's correlation test.

Cut-off values were determined for each serum factor according to the best discrimination between patients and controls regarding optimal values of sensitivity and specificity using the ROC curve analysis. We calculated the area under the curve (AUC) of the tests using ROC curves. p Values < 0.05 were considered to be significant. The report design was adopted from the standards for reporting diagnostic accuracy (STARD) group.[13]

Results

The characteristics of patients are listed in Table 1. The median age of patients was 49 (24–71) years. The majority of the patients was invasive ductal carcinoma (60%), grades I–II (63%) and pre menapousal status (58%).

The mean and median values, standard deviations, and ranges of serum VEGF, angiogenin and TGF-beta 1 levels in patients with breast cancer and the control group are shown in Table 2. The mean serum VEGF concentration in patients compared to controls was not significantly different (0.33 ng/mL vs 0.43 ng/mL, respectively; $p = 0.156$). Likewise, the insignificant change in mean values in patients vs controls was also observed for serum TGF-beta 1 (0.19 ng/mL vs 0.19 ng/mL, respectively; $p = 0.215$) and serum angiogenin (243.24 ng/mL vs 244.5 ng/mL, respectively; $p = 0.976$).

The cut-off levels were chosen by accepting the best sensitivity and specificity levels using ROC curve coordinate points for angiogenin as 0.133 ng/mL, for TGF-beta 1 as 0.21 ng/mL and for VEGF as 2.76 ng/mL. The areas under the curve (AUC) were for angiogenin 42.7% (95% confidence interval: 24.2–61.2), TGF-beta 1 52.8% (95% confidence interval: 35.4–70.2) and VEGF 58.2% (95% confidence interval: 39.9–7.66), respectively. According to these cut-off points, the sensitivity and specificity of angiogenin, TGF-beta 1 and VEGF were calculated in the patients and healthy control groups (Fig. 1).

Spearman's correlation test was used for calculating the correlations. The significant correlation was found between VEGF and angiogenin levels in patients ($r = 0.423$; $p < 0.001$) (Fig. 2). We did not find any correlation between VEGF–TGF-beta 1 and angiogenin–TGF-beta 1 in breast cancer patients ($p > 0.05$).

Table 1
Patients characteristics.

Parameter	n	(%)
No. of patients	90	100
Median age (min–max)	49 (24–71)	
Menapousal status		
Premenapouse	52	58
Postmenapouse	38	42
Clinical stage (AJCC)		
Stage I	32	35.6
Stage II	58	64.4
Histology		
Invasive ductal ca	54	60
Invasive lobular ca	17	18.9
Other	19	21.1
Histological grade		
Grades I–II	63	70
Grade III	27	30
ER status		
Negative	13	14.4
Positive	73	81.1
Unknown	4	4.5
PR status		
Negative	9	10
Positive	75	83.3
Unknown	6	6.7
c-erbB2		
Negative	11	12.2
Positive	19	76.7
Unknown	10	11.1

Discussion

In the present study, we evaluated the serum concentration of angiogenin, TGF-beta 1 and VEGF in breast cancer patients in the early clinical stages. We couldn't find any significantly elevated serum levels of VEGF, angiogenin and TGF-beta 1 in breast cancer patients compared with the healthy controls. Only a significant correlation was found between VEGF and angiogenin serum parameters.

The sources of increased VEGF concentrations in peripheral blood from patients with cancer are not known in detail. Both serum and plasma levels of VEGF have been shown to be of prognostic significance in most studies. Moreover, some researchers recommended that serum or whole blood, but not plasma, should be used in evaluating VEGF in the circulation in cancer patients.[1,14]

Increased VEGF serum levels have previously been reported in smaller and larger series of breast cancer patients.[15,16] Granato et al.[17] verified the potential of VEGF serum levels as non-invasive diagnostic markers. Their results seem to indicate that VEGF serum levels are not useful as a diagnostic tool for breast cancer due to the sets of overlapping for and against values of some previous results

Table 2
The levels of tests according to the groups (mean ± standard deviation; median; range).

	VEGF (ng/mL), $x \pm$ sd, m (min–max)	TGF-beta 1 (ng/mL), $x \pm$ sd, m (min–max)	Angiogenin (ng/mL), $x \pm$ sd, m (min–max)
Patient ($n = 90$)	0.33 ± 0.31, 0.19 (0.062–1.03)	0.19 ± 0.016, 0.18 (0.16–0.2)	243.24 ± 69.42, 244 (142–398)
Control ($n = 75$)	0.43 ± 0.63, 0.18 (0.017–2.27)	0.19 ± 0.07, 0.18 (0.18–0.2)	244.51 ± 77.77, 218 (126–421)
p vs control	=0.156	=0.215	=0.976

Mean (x), standard deviation (sd), median (m), minimum (min), and maximum (max) of values.