Plasma VEGF levels in breast cancer patients with and without metastases

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Abstract. Vascular endothelial growth factor (VEGF) is a key mediator of angiogenesis since it stimulates the formation of new blood vessels. Basic fibroblast growth factor (bFGF) is related to the promotion of endothelial cells into tube-like structures, and it is therefore expected to promote angiogenesis with a greater potency than VEGF. VEGF and bFGF are considered to be biomarkers that predict treatment effectiveness. Elevated plasma VEGF and bFGF levels have been reported in a variety of different malignant tumors, and patients with metastatic disease have also been reported to present with higher serum VEGF and bFGF levels. Other studies have documented controversial results with respect to the prognostic and predictive value of the aforementioned biomarkers. This study aimed to determine the plasma VEGF and bFGF levels in breast cancer patients without metastatic disease compared with breast cancer patients with advanced metastatic disease. The study included 93 patients with breast cancer, 46 without recurrent disease (group A) and 47 with metastatic disease (group B), as well as 21 healthy individuals. The median age was 58 years (range 34-78) for group A and 59 years (range 37-75) for group B. All 93 patients underwent chemotherapy, adjuvant for group A, and adjuvant plus chemotherapy for group B patients with advanced disease. Plasma VEGF and bFGF levels were determined using a quantitative sandwich immunoassay, and samples were tested in triplicate (ELISA). The plasma levels of VEGF and bFGF varied greatly, i.e., from extremely low to extremely high in the two groups, as well as in the healthy individuals. No statistically significant difference was found between the two groups or between the patients and healthy individuals. Data of the present study therefore showed that VEGF and bFGF levels are not valuable biomarkers for predicting treatment outcome.

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Introduction

Certain biomarkers predict the treatment effectiveness of a number of targeting therapies. Molecular biology research has detected a number of protein receptors that regulate certain functions, such as cancer cell development. Vascular endothelial growth factor (VEGF) is a key mediator of angiogenesis since it stimulates the formation of new blood vessels. VEGF is a homodimeric glycoprotein with a molecular weight of approximately 45 kDa (1) and a member of the VEGF platelet-derived growth factor (PDGF) family of structurally related mitogens (2). At least four main VEGF isoforms exist as a result of alternative patterns of splicing (3,4). A variety of factors, including PDGF, FGF, EGF and TNF up-regulate VEGF gene expression (5). Hypoxia also induces VEGF (6).

As with VEGF, 20 members of the basic fibroblast growth factor (bFGF) family, all of which are structurally related to signaling molecules, have been identified. One of the most important FGF isoforms is FGF 2 or bFGF. Since its function is the promotion of endothelial cells into tube-like structures, it is anticipated to promote angiogenesis with a greater potency than VEGF, since is chemotactic and mitogenic for endothelial cells. The FGF isoform modulates embryonic development and differentiation. Furthermore, it stimulates the proliferation of mesodermal and neuroectodermal cells (7-9).

Elevated serum and urine levels of VEGF and bFGF have been reported in patients with a variety of different malignant tumors (19). Patients with metastatic disease presented with higher serum VEGF and bFGF levels when compared to patients with localized disease (10). Subsequently, studies were performed to determine the diagnostic and prognostic (or predictive) value of these biomarkers. Results of these studies showed fluctuations in plasma (serum) VEGF levels, irrespective of the existence of the disease in the case of breast cancer patients (11-14).

Information is scarce regarding the plasma levels of VEGF and bFGF in breast cancer patients with respect to advanced versus non-advanced disease. This study aimed to determine the plasma VEGF 165 and bFGF levels in patients with versus those without recurrent metastatic disease. VEGF and bFGF plasma levels were also examined in healthy individuals, as controls.

Table I. Characteristics of the patients (n=93) and controls (n=21).

	Group A	Group B	Healthy controls
No of patients[a]	46	47	21
Age, years			
Median	58	59	38
Range	34-78	37-75	30-58
Performance status (WHO)			
0	46	15	21
1	-	26	-
2	-	6	-
Disease stage			
I-III at diagnosis	46	-	-
IV	-	47	-
Disease metastasis			
Bone	-	7	-
Liver	-	14	-
Lungs	-	6	-
Multiple	-	20	-

[a]No. of evaluable patients, 93. WHO, World Health Organization.

Materials and methods

Patient eligibility. Eligibility for the study required histologically confirmed breast cancer. All patients had previously undergone surgery, and the primary tumor was excised. Patients with no recurrence after a follow-up of 5-20 years, and patients with recurrent disease within the first 5 years of follow-up were included. Patients with metastases had bidimensionally measurable disease on physical examination, X-rays, computed tomography (CT), WHO performance status (PS) of 0-2, expected survival ≥12 weeks, adequate bone marrow reserves (leukocyte count ≥3500 μl^{-1}, platelet count ≥100.000 μl^{-1}, and hemoglobin ≥10 g μl^{-1}), adequate renal function (serum creatinine ≤1.5 mg dl^{-1}) and liver function (serum bilirubin ≤1.5 mg dl^{-1}) and serum transaminases (≤3 times the upper limit of normal or ≤5 times the upper limit of normal in cases of liver metastases) and age ≥18 years. Patients with a second primary malignancy were excluded. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines (15) and was approved by the hospital institutional ethics review boards. All 93 patients gave their informed consent before entering the study.

Study design and methods

Laboratory work technique. Blood samples were obtained after at least 5 h of fasting. Plasma VEGF and bFGF levels were determined using a quantitative sandwich immunoassay technique (Quantikine; R&D systems) according to the manufacturer's instructions, and all samples were tested in triplicate. Briefly, 100 μl of the sample was added to 100 μl of diluent/well of the ELISA plate and incubated for 2 h at room temperature. After the wells were washed three times, 200 μl of VEGF or bFGF conjugate was added in each well followed by a 2-h incubation. Subsequently, 200 μl of substrate was added to each well, followed by a 25-min incubation at room temperature. The reaction was stopped by the addition of 50 μl stop buffer (2 M sulphuric acid), and the color intensity was measured in each case at 450 nm for VEGF and 490 nm for bFGF using a microplate reader. Corrections for the obtained values were carried out by subtracting the readings of 450 nm or 490 nm from the reading of 540 nm, as indicated by the manufacturer of the kits.

Table II. Comparison of VEGF and bFGF markers between patient group A (no recurrence) and group B (with recurrence).

	Group A No recurrence (n=46) Median (Q1-Q3)	Group B With recurrence (n=47) Median (Q1-Q3)	P-value[a]
VEGF	84.16 (12.38-195.54)	86.33 (18.31-182.30)	0.979
bFGF	73.92 (28.62-142.18)	44.79 (23.13-144.86)	0.541

[a]Mann-Whitney test.

Statistical analysis. Continuous variables were reported as the mean ± standard deviation. Relationships between categorical variables were tested by χ^2 analysis. Yate's correction or Fisher's exact test were used when necessary. The ANOVA test was used to compare mean VEGF and bFGF values between the two groups of patients. $P<0.05$ was considered to be statistically significant. Data were analyzed using SPSS 13.0.

Data were presented as the median and inter-quartile range (Q1-Q3). A comparison of the two groups was carried out by the non-parametric Mann-Whitney test. A comparison of statistical differences was performed between the two groups (group A patients without metastasis and group B patients with metastatic disease).

Results

The study included 93 patients with breast cancer. The patients had initially undergone surgery, where the primary tumor was excised. At the time of examination, 46 patients were without recurrent disease (group A) and 47 had metastatic disease (group B). The median age was 58 years (range 34-78) for group A and 59 years (range 37-75) for Group B. The patients had chemotherapy (adjuvant, for group A) and adjuvant plus chemotherapy for a second time when the disease recurred (group B). Table I shows the patient characteristics. The healthy controls consisted of 21 cancer-free individuals. All 93 patients (46 patients without metastasis and 47 with metastases) were evaluable for the VEGF and bFGF plasma values.

No statistically significant difference was determined between the two groups in either VEGF or bFGF values. The results are shown in Table II. In the healthy individuals, the plasma values of VEGF and bFGF fluctuated in a similar manner to that of the patients. These values were 5-680 with a median of 74.

Discussion

Elevated VEGF expression was reported to correlate with poor prognosis for cancer patients (13-15). It was also suggested that VEGF is a prognostic marker that determines high versus low risk for disease relapse, and whether patients are node-negative or node-positive (14,16,17). Patients with high levels of VEGF in tumors are unlikely to benefit from adjuvant conventional treatments. This may indicate that VEGF has predictive value in the treatment of breast carcinoma (16). VEGF expression correlates with microvessel density, indicating the direct involvement of VEGF in angiogenesis (18). Similarly to VEGF, basic FGF is involved in tumorigenesis, angiogenesis and metastasis (18). The stromal-derived fraction of bFGF is the predominant form in breast tumors (16,18). Studies of either node-positive or node-negative breast tumors reported negative results with regard to the clinical significance of bFGF (15-18). The relationship between tumor microvessel counts and bFGF levels implies no direct involvement between bFGF and angiogenesis. One important point is that bFGF may be one of the multiple factors that synergize with other growth factors in order to enhance angiogenesis (18). In our results, VEGF as well as bFGF plasma levels were shown to be extensively varied and showed no difference in patients with recurrent metastatic disease versus patients without recurrent disease. This extensive variation in plasma values indicates that VEGF and bFGF are not biomarkers with a prognostic and predictive value. Data from the literature presented here are controversial and provide no definite answers regarding the role of VEGF and bFGF values in carcinogenesis and metastasis. Two other studies confirm this conclusion. In one study, high levels of bFGF showed a significantly longer disease-free survival than in patients with a low bFGF level (19). The opposite results were documented in a second study (20). The above-mentioned findings as well as data related to treatment with anti-angiogenic agents (agents that target VEGF) are controversial. In clinical practice, bevacizumab, which targets VEGF, has shown both positive and negative results concerning the prolongation of survival in patients with colorectal cancer (21-24).

In conclusion, our findings as well as the inconsistencies documented in the literature, do not confirm that VEGF and bFGF levels act as convincing biomarkers that assist the prognosis and prediction of breast cancer as well as other types of cancer.

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