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Research Article

Serum Vascular Endothelial Growth Factor as Prognostic Biomarker in Egyptian Breast Cancer Patients

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ABSTRACT

Cancer breast is considered as the most common cancer in Egypt; many studies have demanded that determination of serum vascular endothelial growth factor is a surrogate marker of angiogenesis. In this study we aimed to determine the role of vascular endothelial growth factor (VEGF) levels in comparison to cancer antigen (CA) 15-3 and CEA levels in patients with breast cancer. We measured the serum level of VEGF by ELISA technique in 60 patients with histologically proven breast cancer and compared it with control group (23 healthy female volunteers with no previous history of breast cancer and other cancer-related diseases) to explore the association between this biomarker and clinicopathological parameters. The results demonstrated that serum VEGF, CA15-3 and CEA were significantly increased in all patients compared to control group ($p < 0.001$). VEGF was significantly increased in advanced stage patients compared to early stage patients, also, in patients with tumor size bigger than 2 cm and in patients with ER +ve than those ER-ve patients. Serum VEGF level was significantly correlated with tumor stage, grade, size, CA15.3 and CEA. In conclusion; determination of VEGF might be considered as promising prognostic biomarker that can predict the outcome of breast cancer disease.

Keywords:

INTRODUCTION

Cancer breast is the most common malignancy in women worldwide, it accounts for 23% of the total new cancer. It is the main cause of death in women due to malignancy; this is linked with the statement that the available tumor markers show low sensitivity, and so, the cancer is unnoticed in early stages[1]. There are many factors that are responsible for the development and amelioration of breast cancer. Estrogen therapies, obesity, sedentary life, environmental pollution and genetic changes are correlated with increased risk for breast cancer. Cancer development involves proliferative signal changes, suppressor genes inactivation and suppression of apoptosis allowing continuous cell growth and immortality leading to new angiogenesis, invasion and metastasis formation[2]. Tumor markers are especially useful in monitoring treatment and in the finding the late stage of cancer and prediction of its recurrence. Diagnostic biomarkers in patients with breast cancer include several circulating markers, including cancer antigen (CA) 15-3 and carcinoembryonic antigen (CEA), Their prognostic significance is reinforced by many studies, but these biomarkers have low sensitivity and specificity in early stages of cancer. The new applicants for tumor markers may be cytokines like vascular endothelial growth factor (VEGF)[3]. Oxygen and nutrients are essential for cancer cells growth and activities, these are obtained by new vasculature formed by blood vessels in the process of angiogenesis which is required for tumor progression and metastasis formation[4]. VEGF is the most potent and specific angiogenic factor. VEGF regulates the growth and proliferation of many components of blood vessels. It is essential for angiogenesis regulation[5,6]. VEGF is produced by breast cancer cells and tumor tissue more than normal tissue and it correlates with prognosis and overcome of disease progression[7-9]. This study aimed to determine prognostic significance of vascular endothelial growth factor in comparison with CEA and CA15.3 in serum of Egyptian breast cancer patients, also, to analyze the association between this biomarker and clinicopathological parameters of the patients.

Patients and Methods

This study included 60 female patients with breast cancer entering the Surgical Department at National Cancer Institute, Cairo University. Their age ranged from 44 to 62 years. They underwent modified radical mastectomy or breast-conserving surgery with axillary lymph node dissection. Clinical staging was expressed according to the TNM classification system based on evaluation of findings of physical examination, routine laboratory tests, radiological reports (chest X-ray, liver echography, bone scan and computed tomography) and pathological records. Patients received preoperative metastatic investigation in the form of clinical examination, laboratory investigation as well as radiological investigation to exclude metastatic disease before operation. The size of the tumor was < 2 cm in 21 patients and > 2 cm in 39 patients. All patients had invasive ductal carcinoma, 36 patients had positive lymph

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Table 1: Levels of biomarkers according to clinico-pathological state in breast cancer patients and control groups (mean ± SD).

Factors	No.	VEGF (pg/ml)	CA15-3 (U/ml)	CEA (ng/ml)
Controls	23	84±12.6	18.6 ± 0.6	2.4±0.3
Patients	60			
Menopausal state Pre-menopause	25	314±31.2*	37.5±0.5*	4.3±0.2*
Post-menopause	35	327±22.5*	36.2±1.6*	2.9±1.1
Stage				
I, II	26	209±25.6*	32.6±2.8*	2.7±1.2
III, VI	34	517±54.8* †	39.8±1*†	5.1±1.5*†
Grads				
I	17	158 + 17.5*	23 + 0.8*	2.8 + 1.1*
II	29	221+ 26.6 *	28.2 + 0.7*	3.6 + 0.8*
III	14	518 + 55.1 *a	37.5 + 0.8*a	4.9 + 1.4*a
Tumor size				
<2 cm	21	212±30.1*	25.7±1.3*	3.1±0.7*
>2 cm	39	498±54.6*b	35.6±2.2*b	5.2±0.7* b
Nodal status				
Negative	24	313±26.7*	29.1±3.2*	3.4±0.6*
Positive	36	506±50.2*c	35.2±1.8*	3.9±1.1*
Estrogen Receptor				
Negative	23	243±22.1	37.5±2.2 *	5.2±1.7*
Positive	37	289±24.5 d	31.6±2.3*	3.3±1.4*

*P value <0.05 considered significant compared to control.
†→ P significant as compared to stage I, II. a → P significant as compared to tumor grads. b → P significant as compared to tumor size. c→ P significant as compared nodal status. d → significant as compared ER –ve

Table 2: Correlation between serum VEGF in breast cancer patients and various studied factors.

Prognostic factors	r	P value
Menopausal state	0.214	NS
Tumor stage	0.567	< 0.001
Tumor grade	0.572	< 0.001
Tumor size	0.562	< 0.001
Nodal metastases	0.626	< 0.001
ER status	0.136	NS
CA15.3	0.534	< 0.001
CEA	0.571	< 0.001

P value <0.05 considered significant

node and 24 had negative lymph node. The patients did not receive any treatment before surgery and were not taking hormones or oral contraceptives and were non-smokers. Patients having diabetes mellitus, hypertension, rheumatoid arthritis, cardiac diseases, liver disease or renal disorder were excluded from the study. Group of 23 healthy female volunteers (their age ranged from 40–64 years) with no previous history of breast cancer and other cancer-related diseases served as a control group. The study protocol had been approved by the local Ethics Committee and subjects gave written consent to participate in the present study.

Sampling

Ten ml of fasting venous blood samples were collected from patients before operation and left to clot at room temperature to separate sera after centrifuging for 10 minutes at 3000 r.p.m, Sera were divided into several aliquots and stored at −70◦C until assay. Tumor specimens were obtained after surgery.

Laboratory Methods

-Measurement of serum VEGF by the use Quantikine quantitative human VEGF sandwich enzyme-linked immunosorbent assay done by duplicate (R and D system, Minneapolis, MN).

- Measurement of serum CA 15–3 by using ELISA kit done by duplicate supplied from CIS Bio International.

-Measurement of serum CEA by using ELISA kit done by duplicate supplied from Quorum diagnostics.

-ER (estrogen receptor) and PR (progesterone receptor) in the cytosol fractions were determined by enzyme immunoassay (Abbott Laboratories, Chicago, USA); frozen tissues were obtained from patients after surgery homogenized on ice, the homogenate were filtered and centrifuged for 15 minutes at 4 degrees, the pellet collected centrifuged, the supernatant (cytosol) was collected, the protein concentration was adjusted and then ER and PR were measured.

-Statistical analysis was performed with SPSS software for windows. Data were analyzed using Student's t-test, analysis of variance (ANOVA) was used to address differences between patients and controls. Pearson correlation coefficients were used to test the correlation between variables. P<. 05 considered significant.

RESULTS

The current study was conducted on 60 female patients with breast cancer from Surgical Department at National Cancer Institute, Faculty of Medicine, Cairo University, their age ranged from 44-62 years with the mean of 54 ± 6.8years). They had different stages of cancer according to the TNM classification, and the size of the tumor was less than two cm in 21 patients and more than two cm in 39 patients. All patients had invasive ductal cell carcinoma,

twenty four patients had negative lymph nodes and thirty six patients had positive lymph nodes, while, as regards estrogen receptor twenty three patients were negative and thirty seven patients were positive. Table (1) shows serum levels of the studied biomarkers according to clinicopathological state in breast cancer patients. The mean serum levels of VEGF, CA15.3 and CEA in breast cancer patients were significantly higher in comparison to healthy control group ($p< 0.001$), also VEGF, CA15.3 and CEA were significantly higher in advanced stage patients ($p< 0.001$) in comparison to early stages, also in patients with bigger tumor sizes. VEGF level was significantly increased in patients with ER +ve than those ER -ve patients ($p< 0.001$) while CA15.3 and CEA were significantly increased in ER-ve patients more than ER+ve patients ($p<0.001$). Table (2) shows the correlation between VEGF and various factors; tumor stage, grade, size, CA15.3 and CEA. Serum VEGF was correlated with high grade tumor, tumor size > 2 cm, + ve lymph node, and ER + ve positive hormone receptor status.

DISCUSSION

Angiogenesis may be a target for fighting diseases characterized by either poor or abnormal vasculature. Application of specific compounds that may inhibit or induce the creation of new blood vessels in the body may help combat such diseases. Angiogenesis is a process by which new vessels are formed from preexisting blood vessels, it is important for tumor existence and development; there is significant correlation between angiogenesis and metastasis formation[10]. The current study showed that serum VEGF levels increased significantly in breast cancer patients as compared to the control group, our results were in agreement with Heer et al[11] who stated that tumors induce blood vessel growth by secreting various growth factors and that serum VEGF was increased in breast cancer patients than the control and they reported that the main cause of high VEGF level measured in the serum is the breast tumor tissue. Anti-angiogenic treatment is now a promising measure in cancer treatment, experimental inoculation of VEGF antibodies to animals having cancers leads to tumor regression and growth inhibition, so this treatment can be used as adjuvant therapy to cancer patients[12]. The control of angiogenesis in breast cancer is of significance as a basic pointer of response to therapy and survival as mentioned by Salven et al[13]. Byrne et al[14] also concluded that VEGF has a crucial role in breast cancer progress as it is increased in early and late stages of cancer when compared to normals. In the present study we found a significant correlation between serum VEGF levels and advanced tumor stage, high grade tumor, large tumor size > 2 cm, and + ve lymph node, serum CA15.3 and serum CEA levels. These results were in agreement with Enas et al[15] who found that serum VEGF was strongly linked with grade III tumor, large tumor size > 2 cm, ER – ve status, positive lymph node and +ve HER2-neu. Also, these finding are similar to Spill et al[16] who stated that in cancer breast low serum VEGF level was linked with low cancer stage, low grade tumor, and negative lymph-node status. In a study done by Gnerlich et al[17] on breast cancer patients, they found that "high expression of VEGF was not only associated with large tumor size > 2 cm, but also with metastatic deposits, probably through the growth factor inducing a rich vascular network, and a congruently more nutrition situation for tumor growth". In the current study we found no correlation between ER positivity and serum VEGF; this result was in agreement with Colomer et al[18] who found that there was no significant correlation between estrogen receptor and serum VEGF, they reported as VEGF promoter lacks steroid-responsive elements, the induction of the VEGF gene is thought to occur through indirect mechanisms, including the up-regulation of various oncogenes, but on the other hand our results were in contrast with Heer et al[11] who stated that a correlation was found between serum VEGF and ER positivity, they stated that estrogen could induce VEGF expression and action. In several different experimental studies, VEGF levels in tumor tissue correlate with lymph node status and survival[19-21]. They indicated that endothelial cells are highly sensitive to VEGF inducing its growth and proliferation in vitro[22]. Toi and colleagues[23] reported that high expression of VEGF in breast cancer tissue decreased the survival in these patients and worsen prognosis. In conclusion, our results showed that high VEGF level is associated with disease progression, so determination of serum VEGF can predict the aggressiveness of tumor which can guess the outcome of breast cancer disease. Therefore, it seems from the current study that serum VEGF levels could be promising prognostic value for tumor activity.

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