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ASSOCIATION OF SERUM VASCULAR ENDOTHELIAL GROWTH FACTOR LEVEL WITH ESTROGEN RECEPTORS STATUS IN BREAST CANCER PATIENTS.

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Aim: ***Angiogenesis is essential for tumour growth and metastasis. Vascular endothelial growth factor (VEGF) has been suggested as the major angiogenic factor in breast carcinoma. The aim of the present study was to analyze the relationship between the serum of vascular endothelial growth factor (VEGF) in breast cancer patients and its relationship to various prognostic indices and estrogen receptor status.***
Methods: ***VEGF levels was determined in 45 breast cancer patients and 15 healthy women, using enzyme-linked immunoassay technique***
Results: ***Serum vascular endothelial growth factor (VEGF) levels were detected in 45 patients with breast cancer before surgery and at 3 weeks after surgery. The serum VEGF levels of the cancer patients as a group were significantly elevated compared with those of the controls (P < 0.0005). VEGF levels were elevated in patients with invasive cancer of duct carcinoma, and estrogen receptor (ER)-positive tumors. Post-operatively, VEGF level decreased significantly.***
Conclusions: ***Preoperative serum VEGF detects breast cancer with a sensitivity of 62.2%. The relationship to cancer type and ER status may have future therapeutic implications which deserves more extensive study.***
Keywords: ***angiogenesis, breast cancer, diagnostic tool, serum levels.***

INTRODUCTION

Angiogenesis, the formation of new blood vessels from the existing vascular network, is essential for continued tumor growth and metastasis.[1] One of the most potent and specific angiogenic factors is VEGF, also known as vascular permeability factor and vasculotropin.[2] Vascular endothelial growth factor (VEGF) is heparin-binding molecules with potent angiogenic properties both in vivo and in vitro.

Higher VEGF levels have been found in the serum and urine of patients with different tumor types than in healthy individuals,[3-6] and in the serum of patients with metastatic disease than in those with localized disease.[7-8] Some studies in patients with breast cancer have investigated the potential value of serum VEGF levels for diagnostic purposes[4-6,8,9] or for monitoring the clinical course of disease.[10-13]

In the present study, we analyzed the relationship between preoperative serum VEGF and the various prognostic indices of breast cancer including ER .

PATIENTS AND METHODS

This study was a prospective study involving 45 patients undergoing surgery for breast cancer in surgical department, Mansoura University Hospitals, Egypt. All were women with median age 43.3 years (range, 34–67 years). All patients had histologically confirmed breast cancer.

The control group comprised women who were free from any disease associated with an increased angiogenic activity such as diabetic retinopathy, heart disease or lung disease, which could affect VEGF levels and. Healthy women ranged in age from 35 to 50 years, with a median age of 41.6 years.

A single pathologist reported on the resected tumor specimens using the UICC tumor-node-metastasis (TNM) classification. All patients underwent a metastatic screen consisting of liver US and a chest X-ray. A bone scan was performed on all patients with node-positive or locally advanced disease.

Estrogen receptors were determined in the tumor. Serum VEGF levels were determined in 15 healthy women, and in 45 breast cancer patients.

Immunohistochemical staining for estrogen receptor was applied to coated slides, 4 mμ thick section. Specific monoclonal antibody and envision detection kit from DAKO Corporation, Carpinteria, CA, USA were applied. DAP was applied as chromogen. The nuclear reaction is considered positive, however cytoplasmic reaction is not considered. Ten percent staining preparation may be an acceptable cutoff point for ER status by immunohistochemistry.[14]

Immunoassay

VEGF levels were determined using a quantitative sandwich enzyme immunoassay technique (Quantikine; R&D System, Minneapolis, MN, USA) and all samples were tested in duplicate. The protocols recommended by the manufacturer were used. Briefly, patient plasma was collected using EDTA as anticoagulant and stored at -80°C. one hundred μl assay diluent were added to each well of a microtiter plate precoated with mouse antihuman VEGF monoclonal antibody. Plates were incubated at room temperature for 2 or 3 hours for VEGF. The wells were then washed to remove unbound VEGF, supplemented with 200 μl enzyme-linked anti-VEGF and incubated for 2 hours at room temperature. The wells were washed again, supplemented with antibody-linked enzyme substrates, and left for 25 min at room temperature.

After the addition of 50 μl stop reagent (2 M sulphuric acid), the color intensity in each well was measured at 450 nm for VEGF and using a spectrophotometer (Medical System, Germany). The optical density found in the serum samples was compared with a standard curve of VEGF concentrations and was quantified. The coefficient of linearity, which shows the linear correlation between measured absorbance and known amounts of standards, was at least 0.9 for VEGF. The minimum detectable concentration was less than 9 pg/ml for VEGF, as quoted by the manufacturer.

Statistical Analysis: Statistical analysis was performed with SPSS software for Windows [SPSS (UK) Ltd., Surrey, United Kingdom]. One Way Anova test or Mann-Whitney U test for two independent samples were used. Median values were used to analyze the relationship between the serum levels of VEGF and patient characteristics.

RESULTS

The median serum VEGF in the 15 controls was 164.5 pg/ml (range 0 –339.3 pg/ml). We found that age did not influence the expression of serum VEGF when the controls were grouped according to the decade of their age (P = 0.35).

The serum VEGF levels of the cancer patients as a group were significantly elevated compared with those of the controls (median, 308.7 pg/ml; range 45.7–1083.6 pg/ml; P < 0.0005 Mann-Whitney U test) (Fig. 1) The sensitivity of serum VEGF in detecting any breast cancer was 62.2%, and the specificity was 73.3% using a VEGF level of 241 pg/ml as the cutoff value. This represents the upper limit of the 95% confidence interval (CI) of the mean Table 1. Serum VEGF droped significantly after 3 weeks postoperatively (median, 172.6 pg/ml; range 30.4-422.3 pg/ml; P < 0.0005 Mann-Whitney U test).

Table 1. Specificity and Sensitivity.

	Cancer Patient	**Control**
TEST	positive	negative
Positive (VEGF >241ng/ml)	28	4
Negative (VEGF <241ng/ml)	17	11
Total	45	15

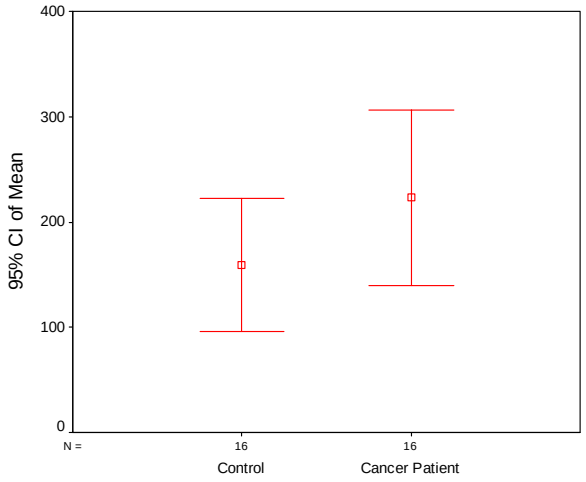

Fig 1. Error bar chart for control group and cancer patients groups.

Patients with histological cancer of ductal origin had a median serum VEGF of 303.8 pg/ml that was significantly

elevated compared with controls (P < 0.0005 Mann-Whitney U test). Patients with lobular carcinoma had serum levels that were not significantly higher than those of controls (median, 187.1 pg/ml; P = 0.76 Mann-Whitney U test). The elevation of VEGF levels in patients with ductal cancer (median 303.8 pg/ml) compared with those in patients with lobular carcinoma (median 178.1 pg/ml) was also significant (P = 0.01; Mann-Whitney U test (Fig. 2).

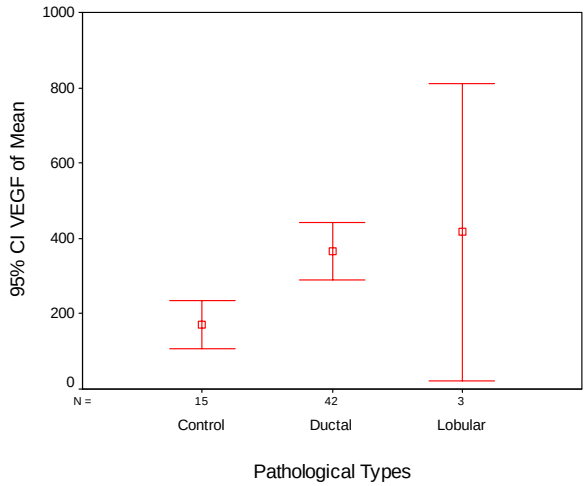

Fig 2. Error bar chart for pathological types

ER-positive tumors had serum VEGF levels (median, 346.5 pg/ml) that were significantly elevated when compared with those of controls (P < 0.0005 Mann-Whitney U test) and patients with ER-negative tumors (median, 194.2 pg/ml; P = 0.05 Mann-Whitney U test). Serum VEGF in ER-negative tumors was not significantly elevated compared with controls (P = 0.24; (Fig. 3).

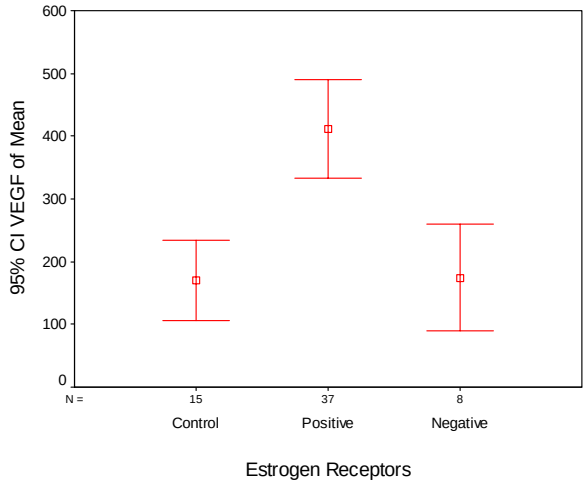

Fig 3. Error bar chart for Estrogen Receptors

Serum VEGF was elevated in all UICC stages compared with controls. Levels in patients with stage IV tumors (median 637.4 pg/ml) were significantly elevated as compared with patients with stages I, II, and III tumors (median, 280.6, 297.3, and 304.5 pg/ml, respectively); (Fig. 4) P= 0.001 One Way Anova test) . Within each stage, the serum VEGF levels in ductal carcinomas and ER-positive tumors were significantly elevated, whereas patients with lobular and ER-negative cancers had serum VEGF levels comparable to those of controls. Serum VEGF levels did not show any significant value with the tumor grade (P = 0.22 One Way Anova test & (Fig. 5).

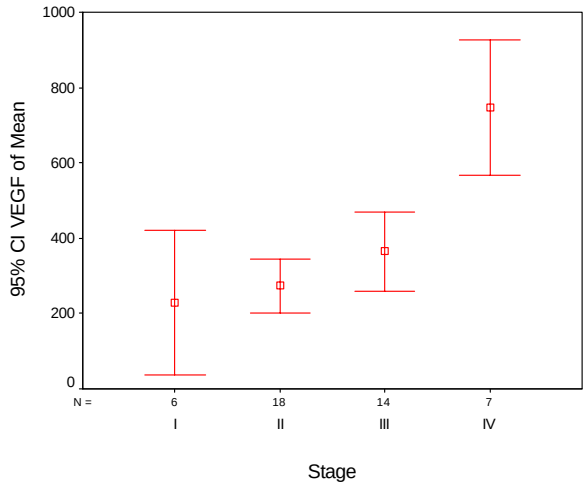

Fig 4. Error Bar Chart comparing 95% CI of VEGF of mean and stage of the disease.

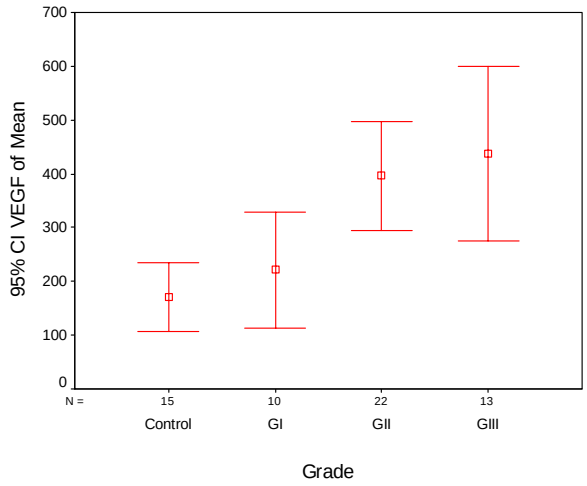

Fig 5. Error Bar Chart comparing 95% CI of VEGF of mean and Grading (I = well differentiated. II = moderate differentiated, III = poor differentiated).

Serum VEGF levels showed a significant correlation positive lymph nodes (P = 0.001 Mann-Whitney U test) (Fig 6), ER status (P= 0.009 Mann-Whitney U test), and the absence or presence of intratumoral lymphatic or blood vessel cancer cell permeation (P = 0.001 Mann-Whitney U test (Fig 7) but did not show any significant value, or hisopathology (P= 0.24 Mann-Whitney U test).

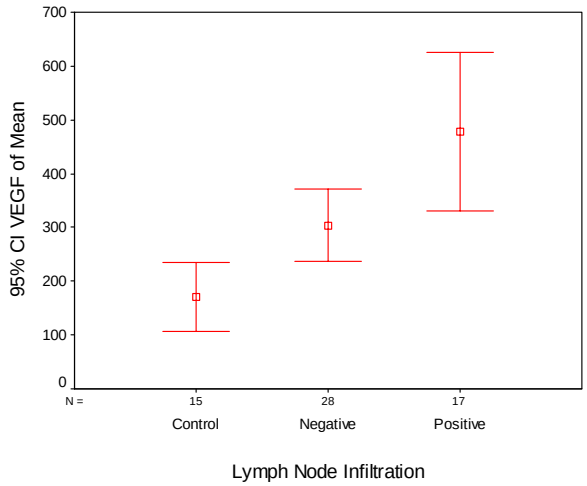

Fig 6. Error Bar Chart comparing 95% CI of VEGF of mean and lymph node infiltration with disease.

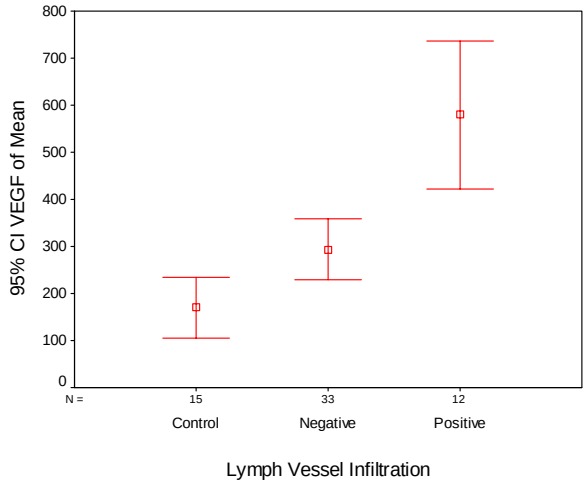

Fig 7. Error Bar Chart comparing 95% CI of VEGF of mean and Lymph Vessel Infiltration

DISCUSSION

VEGF is expressed by a wide variety of tumors, both in vitro and in vivo. Previous studies showed that preoperative levels of VEGF in the serum can predict the stage of colorectal cancer.[15] In breast cancer, intratumoral VEGF and microvessel density significantly correlate with decreased relapse-free survival.[16]

VEGF may exist in one of four forms, namely, VEGF165 (the most abundant form, soluble, and tissue bound), VEGF121 (soluble), VEGF189 (bound), and VEGF206 (extremely rare, bound form). Each of these forms has similar biological activity. The ELISA kit used to detect VEGF in the serum in this study is specific for VEGF165 but will also detect VEGF121. Thus, the detected levels are indicative of the total circulating level of VEGF.[4]

It has been shown that other mediators of neovascularization including interleukins, oncogenes, and some growth factors may produce their effects by altering the expression of VEGF, suggesting that VEGF may be the final common pathway for all pathological in vivo angiogenesis.[2]

Cellular sources of VEGF, other than tumor cells, are multiple. Most of these sources are involved in normal adult tissue repair and remodeling processes. Mast cells and muscle cells are an important source of VEGF (17-18) . Fibroblasts, PMN cells, and monocytes (MOs) are sources of VEGF in the wound-healing process.[19-20] Multiple factors, including hypoxia, nitric oxide, and inflammatory cytokines such as MCP-1,[21] interleukin 1, and interleukin 6, are involved in controlling VEGF expression in normal tissue repair and remodeling events.[22-24] A decrease in VEGF serum levels was shown in our study also observed by other investigators,[4,7,25] and a tendency of VEGF to increase after breast cancer surgery[26] have been reported. These controversial results could perhaps be ascribed to the lack of standardization of the pre-analytical phase related to the serum separation time and the clotting temperature, which can influence platelet activation and, consequently, compromise reliability and reproducibility of the determinations.[25,27]

We have shown that serum VEGF has a sensitivity of 62.2% in detecting breast cancer, with a specificity of 73.3%. Thus, there may be a place to add serum VEGF to the preoperative diagnostic tools, especially in cases of difficult decisions between benign changes and DCIS on mammography, and in agreement with previous results.[6,8] In contrast with Other studies found that VEGF serum levels are not useful as a diagnostic tool for breast cancer due to the considerable overlapping of the sets of values observed in healthy women and in patients.[28-29]

Angiogenesis inhibition is a target for anticancer therapy. In this scenario, the definitions of the actual cellular producers of VEGF in quantities sufficient to promote tumor growth and of the molecular mechanisms involved in stimulating those cells to produce VEGF are fundamental.

This study showed a correlation between serum VEGF and ER positivity. VEGF expression was significantly higher in ductal tumors and, paradoxically, inversely related to the grade and the ER level, as already observed by other authors.[30-31] The up-regulation of VEGF by estrogen has been shown in the rat uterus and human endothelial cancer cell lines.[32-33] Also the same observation have noted with the breast cancer cell line MCF-7.[6] Because the 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. VEGF gene contains consensus estrogen response elements both at the 5' and 3' ends.[34] In this context, the activation protein transcription factor complexes AP-1 and AP-2 have been implicated. Various factors including estrogen, protein kinase C, and cAMP can induce the expression of these gene complexes, which in turn can up-regulate VEGF expression.[35] In ER-positive cell lines, estrogen activates the c-Src tyrosine kinase, which can then induce VEGF expression.[36] The presence or absence of the ER depends on the specific genetic activity of the individual tumor, and ER positivity implies a greater likelihood of estrogen responsiveness of the tumor. Thus, the genetic status of a tumor may determine whether or not estrogen can stimulate VEGF expression.[33] ER-positive tumors have a better prognosis because it is more differentiated and capable of expressing the receptor, or due to increase their responsiveness to hormones. So antiestrogen therapy improve the prognosis of ER positive tumors through decrease the angiogenic potential and thus of these tumors. Tamoxifen, the most commonly used antiestrogenic adjuvant drug, is known to be antiangiogenic.[37]

Previous study of serum VEGF in colorectal cancer had shown good correlation between VEGF levels, cancer stage, and nodal status.[15] Such a correlation has been demonstrated in the present work. In node-negative breast cancer patients, VEGF is a strong independent predictor of relapse-free[38-39] and overall survival.[39-40] The correlation of serum VEGF with ER status would appear to support this. Long-term follow-up studies are required to answer the questions of whether preoperative serum VEGF levels are of prognostic significance, as has been shown for tumor VEGF levels, and whether serum VEGF will be useful in detecting early recurrence in breast cancer.

In conclusion, this study shows that serum VEGF is raised in patients with breast cancer. More importantly, the relation of VEGF with cancer type and ER status may not only throw light on tumor biology but may also have therapeutic implications in the future.

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