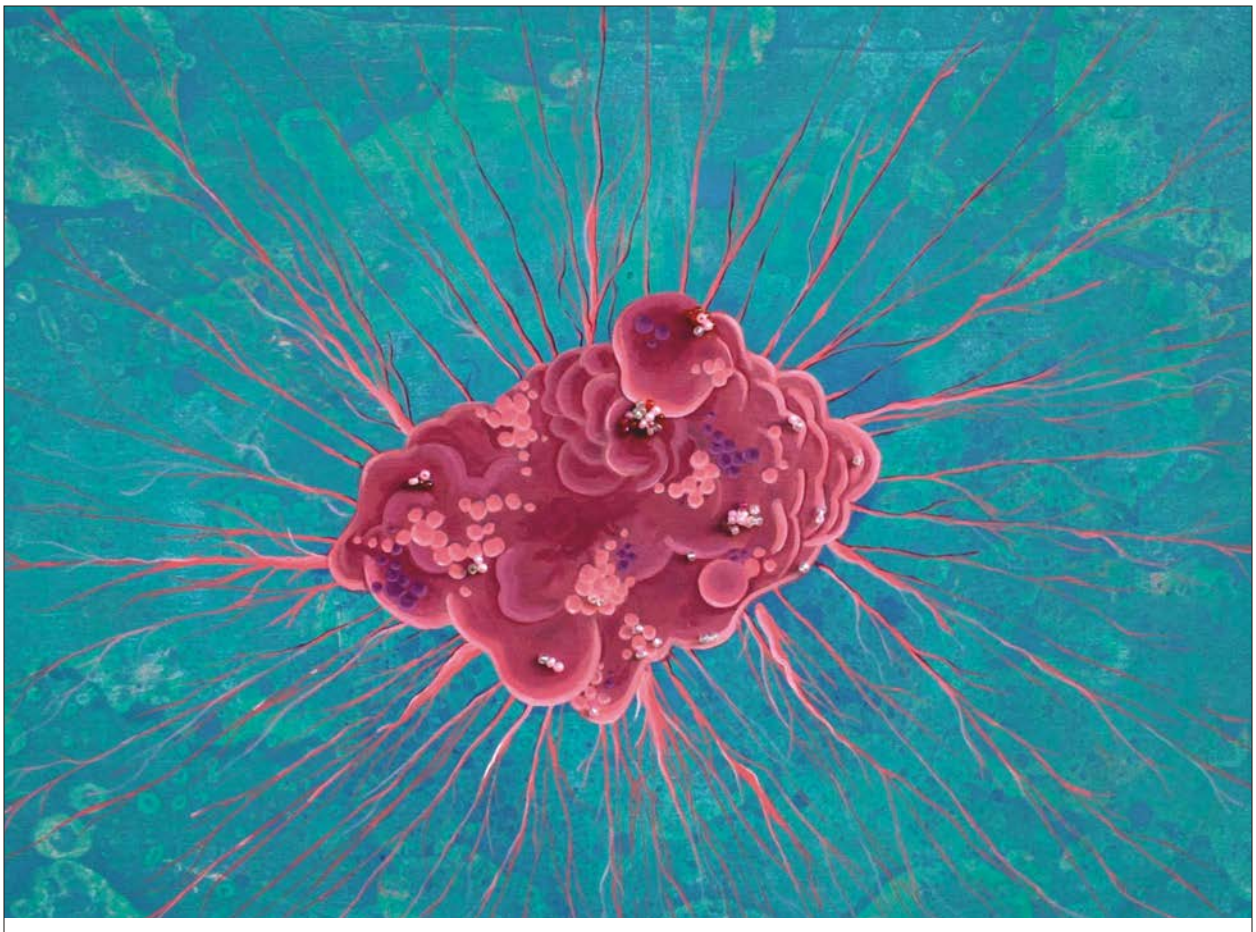

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Research Article
Expression of Vascular Endothelial Growth Factor Protein in Both Serum Samples and Excised Tumor Tissues of Breast Carcinoma Patients

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Abstract

Background and Objective: This study examined the correlation between immunohistochemistry (IHC) expression of the Vascular Endothelial Growth Factor (VEGF) in primary breast carcinoma tissue and its concentration in blood samples of the same patients preoperatively and to the other established clinicopathological parameters namely, tumor size, grade, lymph node status, Estrogen Receptor (ER), Progesterone Receptor (PR) status and human epidermal growth factor receptor 2 (Her2-neu) score. The current study also stressed on whether the ELISA detection is more sensitive and effective in diagnosis of breast cancer. **Materials and Methods:** Serum concentration of VEGF was measured using Enzyme Linked Immune Sorbent Assay (ELISA) in 92 primary breast cancer patients and compared with 25 benign breast disease patients. Also, tissue expression of VEGF was measured by immunohistochemistry. **Results:** Serum VEGF levels were significantly elevated in patients with malignant breast cancer ($p = 0.000$). The median serum level in the malignant group was 579 pg mL^{-1} and in the control group 200 pg mL^{-1}. On the other hand no correlation was found between concentrations of serum VEGF and clinicopathological parameters. A significant association was showed between VEGF expression and lymph node metastasis, tumor size more than 2 cm and Her2-neu status ($p<0.0001$). No significant association was found between VEGF and patient age, histology, grade, ER and PR status ($p>0.05$). All positive cases for VEGF with strong positivity (score ++) were grade 2 and 3. **Conclusion:** The VEGF is overexpressed in breast carcinomas compared to the benign breast disease. Tissue expression of VEGF can be used as a prognostic marker due to the significant association with the large tumor size, lymph node metastasis and positive Her2-neu status. Tissue expression of VEGF can be used as a prognostic marker due to the significant association with the large tumor size, lymph node metastasis and positive Her2-neu status.

Key words: Angiogenesis, VEGF, immunohistochemistry, enzyme linked immune sorbent assay, breast carcinoma, tumor grade, prognosis, HER2-neu

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Breast cancer is the most common female cancer both in the developed and less developed world with nearly (246,660 newly estimated invasive breast cancer cases are expected to be diagnosed in USA women in 2016[1]. Breast cancer is also the most common cause of cancer death among women (522000 deaths in 2012)[2]. In Egypt, breast cancer ranked first among cancers in females representing 32.0% of all newly diagnosed cancers and ranked second among cancers of both sexes (15.4%) following liver cancer (23.8%)[3].

Like other solid tumors, breast cancer requires an independent blood supply to grow and metastasize[4]. This process called tumor angiogenesis, which controlled by several growth factors secreted by tumor cells and tumor surroundings which considered as a key indicator of prognosis and response to therapy[5]. For several years, the only method to assess angiogenesis was the counting of microvessels in the tumor specimens stained for an endothelial marker, the so called microvessel density (MVD)[6]. However, this method relies on the subjective evaluation of individual vessels by the observer. It has not been shown to be a reliable measurement to guide anti-angiogenic therapy which is subjected to the same kind of inter and intra-observer variability[7].

For this purpose, the identification of angiogenic factors is essential. The best known and the most efficient angiogenic factor is Vascular Endothelial Growth Factor (VEGF), member in the VEGF family[8], which is a sub-family of the platelet-derived growth factor family. It acts with its own VEGF receptors[9]. The VEGF is a 46 kDa dimeric heparin binding glycoprotein and its gene is located on chromosome 6p21.3. Many studies have reported that VEGF is overexpressed in breast or in situ carcinoma and is associated with a more aggressive phenotype[8,10].

The three most commonly used methods for measuring VEGF are blood-based quantification using Enzyme Linked Immune Sorbent Assay (ELISA), tissue-based immunohistochemistry (IHC) and tissue-based mRNA measurement[11,12].

Gene study is more sensitive but less specific to distinguish among different cells. It may be contaminated by other cells such as macrophages. In addition, it is complex and inconvenient for routine clinical use[13]. The IHC detection of VEGF is essential in routine diagnosis and research, because it is relatively inexpensive, rapid and allows single cell analysis combined with cell morphology[14]. The ELISA tests are considered highly sensitive, specific and not needing radioisotopes or a costly radiation counter[11].

This study examined the IHC expression of the VEGF in primary breast carcinoma tissue and the results were correlated with the concentration of VEGF in blood samples of the same patients preoperatively and to the other established clinicopathological parameters namely, tumor size, grade, lymph node status, ER, PR status and Her2-neu score. The current study also stressed on whether the ELISA detection can be used as a substitution of IHC tissue expression.

MATERIALS AND METHODS

The study included 100 female patients with non-metastatic breast carcinoma who admitted to National Cancer Institute, Cairo University in the period between December, 2013 and August, 2015. Our study design was approved by Medical Research Ethical Committee, National Research Center, Cairo, Egypt (Approval No. 14-031). An informed consent was taken from each participant before enrollment in the study. Most of our cases (65) were initially diagnosed by fine needle aspiration cytology, 22 cases by core biopsy and 13 cases by excision biopsy prior to surgery. Selected patients were subjected to breast surgery which was either radical or conservative surgery.

All patients had undergone full clinical examination, routine laboratory investigations: Complete blood count, liver and kidney function tests, chest x-ray, mammography, breast and abdominal ultrasonography and bone scan.

The criteria for selecting the patients were: (1) Presence of breast lump which diagnosed as breast carcinoma, (2) Availability of collected venous blood samples of the same patients, (3) No systematic disease such as diabetes mellitus, hypertension, chronic inflammatory disease, liver, renal or heart failure, (4) No distant metastasis and (5) No neoadjuvant therapy.

Control group: The control group consisted of 25 patients with benign breast diseases who were matched with the patients group in terms of age and were also subjected to the appropriate breast surgery.

Blood sampling: Venous blood samples were collected from malignant and control cases. Within 30 min, the sera were separated by centrifugation at 3000 rpm for 10 min after a minimum time span of 30 min and serum were removed, aliquoted and stored at -80^{o}C until further processing.

Measurement of circulating VEGF level: Serum concentration of VEGF was measured using a commercially available sandwich ELISA according to the manufacturer's instructions, the kit was purchased from R and D Systems (Abingdon, UK) catalog number DVE00. The serum VEGF concentrations were

scored as low (negative) if VEGF mean was less than or equal to the 95th percentile of the normal controls mean or otherwise scored as high (positive)[7,15].

Histopathological examination: The excised tumors and breast tissues were sent to the Pathology Department for final histopathological examination and diagnosis. Tumor tissue as well as benign breast control tissue were preserved in formalin and embedded in paraffin wax. Sections were stained with routine hematoxylin-eosin staining and diagnosed according to the criteria of the World Health Organization[16] and graded according to the modified Scarff-bloom and Richardson method[17]. Patient's age, tumor type, size, grade, nodal status, Estrogen Receptor (ER) Progesterone Receptor (PR) and Her2-neu status were reported in the histopathological reports.

Immunohistochemical (IHC) staining and evaluation: For each case, the most representative paraffin block, containing the main bulk of tumor were selected for IHC staining. Four micrometer thickness additional serial sections were placed onto plus-coated slides. The sections were deparaffinized with xylene and rehydrated with graded concentrations of ethanol and water. Endogenous peroxidase was blocked by incubation in methanol with 0.3% hydrogen peroxide for 30 min at room temperature. Heat-induced epitope retrieval was performed for 20 min. After washed in Phosphate Buffered Saline (PBS) and incubated in 10% normal horse serum for 10 min at room temperature to reduce non-specific binding, the slides were subjected to IHC staining using a streptavidin-biotin peroxidase according to the manufacture's protocol using monoclonal mouse anti-human VEGF antibody (clone VG1, M7273, Dako Cytomation, Denmark) at a 1:50 dilution. After visualizing the reaction with 3, 3'-diaminobenzidine (DAB) using DAKO EnVision/HPR, K4004 and the slides were counterstained with haematoxylin solution.

Breast invasive duct carcinoma tissue previously known to be positive for VEGF was used as positive control for VEGF. Omission of the primary antibody was used as negative control. Control samples were included in each slide run. All controls yielded appropriate results.

Staining of slides was assessed independently by the two pathologists. The VEGF cytoplasmic staining of tumor cells was scored by combining the percentage and the intensity of stained tumor cells among the total malignant cells. The percentage of stained cells was assessed using a 4-point scale: 0 if less than 10% of tumor cells were stained, 1 if 10-25% of tumor cells were stained, 2 if 25-50% were stained and 3 if more than 50% were stained. The staining intensity was also graded using a 4-point scale; 0: No staining, 1: Light yellow, 2: Brown and 3: Dark brown. The combined score was calculated by adding the individual scale of the percentage of positive cells and the intensity of stained cells (range 0-6). The combined score was assessed as follows: 0-2: Negative staining (-), 3 and 4: Positive staining (+) and 5 and 6: Strong positive staining (++)[18,19].

Statistical analysis: Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 16. Non parametric variable were selected. Categorical and continuous variables were analyzed using chi square, Mann-Whitney U tests and Kruskal-Wallis tests. Continuous variables were modeled stratifying by median.

Correlation between variables was analyzed using Spearman's rank correlation coefficient. Two-sided tests of significance were performed. The p-values lower than 0.05 were considered to be associated with statistical significance.

RESULTS

By light microscopy, cases which diagnosed as infiltrating ductal carcinoma were 82 cases. Eighteen cases were associated with foci of intraductal component, minor or major. Large tumor size (pT2 or pT3) and tumor with chest wall or skin infiltration (pT4) were seen in 90 cases. Among the studied cases, 80 cases were G2, 18 cases were evaluated as G3 and 2 cases as G1. Fifty two cases showed lymph nodes metastasis (pN1, pN2 and pN3) while 48 cases were negative for node metastasis. The ER, PR and Her2-neu were positive in 20, 24 and 32 cases respectively.

Among the 100 studied non metastatic breast carcinoma cases, 8 sera samples were undergone autolysis and hence were unavailable for serological evaluation. The VEGF was measured by ELISA in the serum of patients with primary breast carcinoma (n = 92) and controls with benign breast lesions (n = 25), both groups were age matched. Serum VEGF levels were significantly elevated in patients with malignant breast cancer compared to control group (p = 0.000) as assessed by Mann-Whitney U test (Table 1). The median

Table 1: Serum VEGF level (pg mL^{-1}) in studied groups

	Study groups		
Markers	Benign control group (*No. = 25)	Primary breast cancer (No. = 92)	Mann-Whitney test (p-value)
VEGF (pg mL^{-1})			
Minimum	41.0	27.0	0.000
Maximum	498.0	1837.00	
Median	200.00	579.00	

*Number of cases

serum level in the malignant group was 579.00 pg mL^{-1} and in the control group 200 pg mL^{-1} as assessed by Mann-Whitney U test (Fig. 1).

Serum VEGF levels in patients with malignant disease was also examined according to tumor stage. The levels of VEGF in patients with different stage of disease showed no significant difference, pTl = 1243, pT2 = 510.5, pT3 = 432.5 and pT4 = 810 pg mL^{-1}. There was no significant difference in serum VEGF levels between patients who were node positive and node negative (Mann-Whitney U>0.05). There was also no significant difference between VEGF levels in patients who were ER, PR and HER2-neu positive and negative (Mann-Whitney U>0 05) as shown in Table 2.

Immunostaining with VEGF showed positive reaction in 60 of our studied cases. Tumor cells show granular cytoplasmic staining pattern. Using VEGF scoring system (18, 19), it was found 30 cases with score ++ (Fig. 2, 3), 30 cases with score + (Fig. 4) and 40 cases with score (-) (Table 3). The VEGF was negative in cases with benign breast disease (fewer than 10% of cells were positive for VEGF). The surrounding ductal epithelial cells located away from the

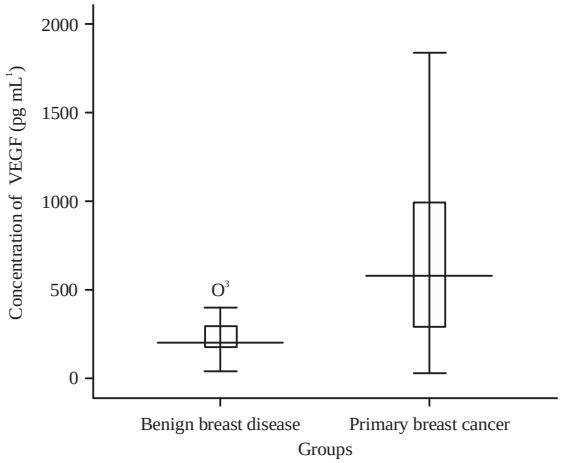

Fig. 1: Serum levels of VEGF in patients with malignant breast disease (No. = 92), patients with benign breast lesions (No. = 25). Data is expressed as median

Table 2: Relation between VEGF serum expressions and clinicopathological characteristic of the 92 cases

Characteristics	No. of cases	VEGF median (pg mL^{-1}) (IQR)	p-value
Age			
<50 years	50	441 (292.5-848)	0.235
≥50 years	42	739.5 (308-1100)	
Histology			
IDC*	80	530 (283-994)	0.897
ILC**	8	807 (620-997)	
Mixed IDC and ILC	4	404 (356-615)	
Tumor size			
pT1	10	1243 (433-1277)	0.399
pT2	62	510.5 (398-896)	
pT3	14	432.5 (89-924)	
pT4	6	810 (536-1060)	
Grade			
1	2	885	0.694
2	76	579 (294-997)	
3	14	495 (318-853)	
Lymph nodes			
pN0	44	642 (308-1186)	0.536
pN1	4	398	
pN2	30	530 (256-818)	
pN3	14	531.5 (423-1168)	
ER* **			
-ve	78	579 (291-1100)	0.978
+ve	14	487 (441-826)	
PR#			
-ve	72	491 (301-976)	0.990
+ve	20	632 (337-947)	
HER2-neu##			
-ve	64	605.5 (301-1049)	0.438
+ve	28	510.5 (2623-810)	

*Invasive ductal carcinoma, **Invasive lobular carcinoma, ***Estrogen receptor, #Progesterone receptor, ##Human epidermal growth factor receptor, VEGF: Vascular endothelial growth factor, IQR: Inter-quartile range

Table 3: Distribution of VEGF scores related to the clinicopathological characteristics of the 100 studied cases

		VEGF score		
Clinicopathological	Total	0 (No. = 40)	+ (No. = 30)	++ (No. = 30)
Age				
<50 years	52	20	18	14
≥50 years	48	20	12	16
Histology				
IDC	82	32	26	24
ILC	10	6	2	2
Mixed IDC and ILC	8	2	2	4
Tumor size				
pT1	10	10	0	0
pT2	66	24	28	14
pT3	16	2	2	12
pT4	8	4	0	4
Grade				
1	2	0	2	0
2	80	36	26	18
3	18	4	2	12
Lymph nodes				
pN0	48	36	8	4
pN1	4	0	2	2
pN2	32	4	16	12
pN3	16	0	4	12
ER				
-ve	80	36	16	28
+ve	20	4	14	2
PR				
-ve	76	32	16	28
+ve	24	8	14	2
HER2-neu				
-ve	68	40	22	6
+ve	32	0	8	24

ER: Estrogen receptor, PR: Progesterone receptor

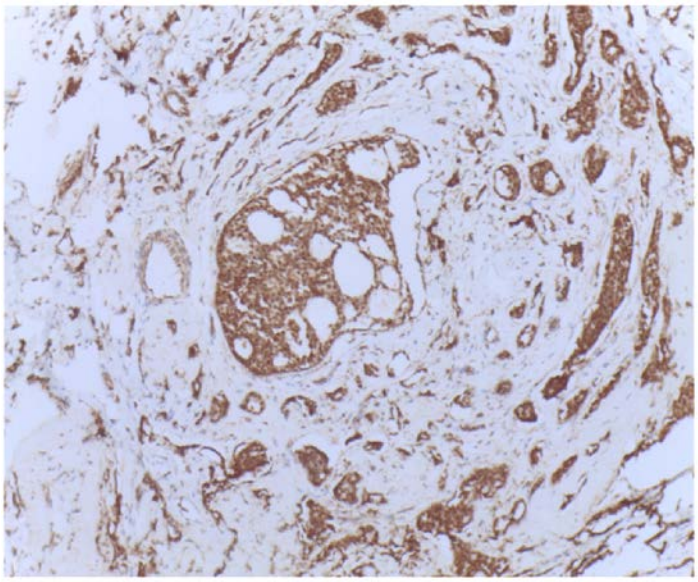

Fig. 2: Invasive duct carcinoma with intraductal component show very strong expression (score ++) (IHC-VEGF×200)

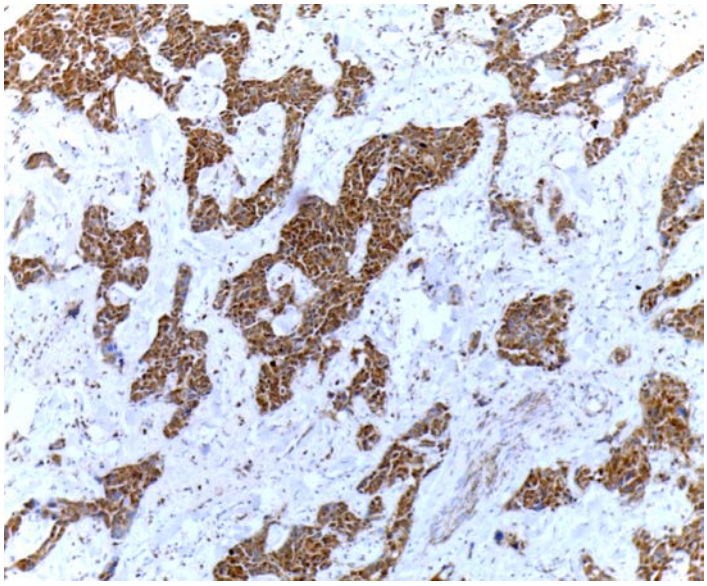

Fig. 3: Grade III invasive duct carcinoma show very strong expression (score ++) (IHC-VEGF×100)

malignant proliferation were negative. Cells of the tumor stroma were also negative. Most invasive carcinoma cases with foci of intraductal component (15/18) showed significant expression of VEGF in the *in situ* foci.

The association between VEGF tissue expression and the clinicopathological parameters are shown in Table 4. Statistical analysis showed a significant association between VEGF expression and lymph node metastasis, tumor size and Her2-neu status. Forty eight out of 52 cases (92.3%) with lymph node metastasis showed positive reaction for VEGF and the correlation was significant ($p<0.0001$). A significant correlation was found between the expression of VEGF and the large size of the tumor, more than 2 cm in size ($p = 0.006$). A positive association was detected between VEGF expression and Her2-neu positive cases ($p<0.0001$). No significant association was found between VEGF and patient

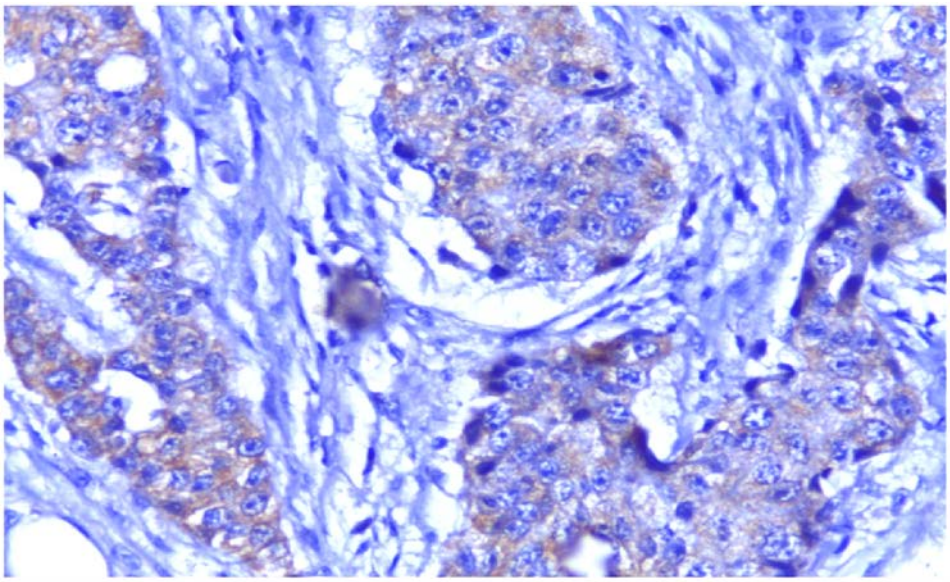

Fig. 4: Grade II invasive duct carcinoma show strong expression (score +) (IHC-VEGF×400)

Table 4: Relation between VEGF tissue expressions and clinicopathological characteristic of the 100 studied cases

		Tissue VEGF		
		Studied cases (IHC)		
Clinicopath.	Total	Positive (No. = 60)	Negative (No. = 40)	p-value
Age				
<50 years	52	32	20	0.817
≥50 years	48	28	20	
Histology				
IDC	82	50	32	0.592
ILC	10	4	6	
Mixed IDC and ILC	8	6	2	
Tumor size				
pT1	10	0	10	0.006
pT2	66	42	24	
pT3	16	14	2	
pT4	8	4	4	
Grade				
1	2	2	0	0.257
2	80	44	36	
3	18	14	4	
Lymph nodes				
pN0	48	12	36	0.000
pN1	4	4	0	
pN2	32	28	4	
pN3	16	16	0	
ER				
-ve	80	44	36	0.279
+ve	20	16	4	
PR				
-ve	76	44	32	0.740
+ve	24	16	8	
HER2-neu				
-ve	68	28	40	0.000
+ve	32	32	0	

IDC: Invasive ductal carcinoma, ILC: Invasive lobular carcinoma, ER: Estrogen receptor, PR: Progesterone receptor

Table 5: VEGF serum concentration results related to the IHC expression by the tumor

	Serum		
VEGF	Positive	Negative	Total
IHC			
+ve	48 TP	6 FN	54
-ve	36 FP	2 TN	38
Total	84	8	92

TP: True positive cases, FN: False negative cases, FP: False positive cases, TN: True negative cases, VEGF: Vascular endothelial growth factor, IHC: Immunohistochemistry

age, histology, grade, ER and PR status (p>0.05). All positive cases for VEGF with strong positivity (score ++) were grade 2 and 3 and no cases were grade 1. Most of grade 3 VEGF positive tumor (14/18) showed a significantly stronger VEGF expression (score ++). Fourteen out of 20 ER positive tumors expressed score+VEGF.

Correlation between VEGF IHC tissue expression and its serum concentration: Among the 100 studied breast carcinoma cases, 92 cases were available to examine the relation between tissue expression and serum level of VEGF. Fifty four cases (58.7%) showed accurate correlation and 38 cases (41.3%) revealed no significant correlation was found between VEGF tissue expression and its serum level (p = 0.632) (Table 5).

The sensitivity of VEGF serum level when compared with tissue expression was 88.9% with 95% Confidence Interval (CI) of 77.37-95.81%, specificity was 5.3% with 0.64-17.75% CI, positive predictive value and negative predictive value were

57.1% with 45.88-67.89% and 25% with 3.19-65.09%, respectively. The accuracy was 54.3%. No significant correlation was found between serum VEGF level and tissue expression of the same cases ($p = 0.866$ and $r = 0.022$).

DISCUSSION

Angiogenesis is an essential step for breast cancer growth, progression and dissemination. The assessment of angiogenic factors has a special importance in selecting patients who may gain benefit from anti-angiogenic therapies. Measurement of circulating and locally expressed angiogenic cytokines and VEGF might be useful in this respect[20]. The VEGF has been recognized as the strongest angiogenic factor implicated[21]. A high expression of VEGF is determined in most cases with poor prognosis[22]. This is may be attributed to its role in angiogenesis and maintaining the integrity of large blood vessels[23], which would explain the presence of lower levels of VEGF in people with benign tumors than others with malignant one[24]. Since previous studies[23-26], reported variation of VEGF expression by IHC in breast cancer as well as uncertain relation with clinicopathological features of breast cancer, we studied serum and IHC expression of the VEGF in nearly 100 Egyptian primary breast carcinoma cases and its relation to some clinicopathological parameters.

The study showed statistical difference between serum concentration of VEGF in benign breast lesions and primary breast cancer patient. This may raise the possibility of using VEGF in differentiating between patients with malignant and benign breast tumors. On the other hand no correlation was found between concentrations of VEGF and the patient's age, size of the primary tumor, metastasis to lymph nodes, histological type and grade. Similar results were obtained by other authors. Valkovic *et al.*[27], Shahi *et al.*[28] and Hina *et al.*[29] found no correlation between VEGF concentrations and tumor size, metastasis to regional lymph nodes or cancer stage[27-29]. On the other hand, significantly higher VEGF concentrations were found in the patients with metastatic cancer as compared with a group of patients with an advanced stage of local or regional cancer[22].

The VEGF tissue expression was detected in 60% of these studied cancer cases which was in agreement with previous reports[30,31] and more than the positivity rate reported by Schoppmann *et al.*[32]. However, the rate of expression was low compared to other studies[10,14,19,26,29]. These differences may be attributed to different VEGF clones used for immunostaining, difference in the systems used for scoring, technical skills and to the relatively small sample size in some studies[10,19].

The difference was significant between cancer cases and control cases regarding tissue expression of VEGF. This finding was expected as VEGF expression has no or limited role in the benign lesions. This was matched with other studies which were also performed on breast carcinoma[4,7,10,31]. This can confirm the concept that this growth factor is involved in the breast carcinoma development and thus can be used to differentiate between malignant and benign breast cases[10].

Cells of the tumor stroma were negative to VEGF immunoreactivity. This result was inconsistent with others who reported expression of VEGF not only in carcinoma cells but also in inflammatory cells, endothelial cells and fibroblast[10,27]. They concluded that these cells can be a potential source of more VEGF.

Most invasive carcinoma cases with foci of intraductal component (15/18) showed significant expression of VEGF in the *in situ* foci, indicating that the non-invasive carcinoma is capable of inducing angiogenesis. Others in their study on 200 cases of *in situ* carcinoma found a higher VEGF expression in pure DCIS as compared to DCIS with concomitant invasive carcinoma and the high expression was considerably more pronounced in the low grade *in situ* carcinoma as compared with high grade *in situ* carcinoma. They concluded that detection of these angiogenic markers in pure DCIS may help in identifying subset with a potentially higher risk of progression that could benefit from targeted antiangiogenic therapy[33].

In this study, VEGF tissue expression was significantly associated with large tumor size, more than 2 cm ($p = 0.006$) confirming the dependence of tumor expansion on angiogenesis. This finding was in accordance with the results of others[7,25]. In contrast, no significant correlation was found in several previous studies[4,7,10,27]. Comsa *et al.*[26] reported an inverse correlation between VEGF expression and tumor size, the expression was found to be positively associated with early tumor size compared to large size[26].

The VEGF expression was significantly associated with lymph nodes metastasis in the studied cases, which indicates that VEGF expression is associated with tumor progression, spread and poor prognosis, probably by stimulating angiogenesis. This finding was in agreement with the results from previous studies[4,27,31]. In contrast, no correlation was detected by others[7,10,26].

The VEGF expression in our cases was positively correlated with Her2-neu positive expression explaining the aggressive phenotype associated with VEGF positive tumors. This finding is compatible with a study done by Schoppmann *et al.*[32]. They reported that inhibiting HER2-neu

may reduce tumor progression by blocking VEGF mediated tumor cell proliferation and metastasis. On the other hand, other authors did not obtain a significant relationship between VEGF expression and Her2-neu status[34].

In this study, it was also found that no association was found between VEGF expression and histological types of breast carcinoma cases. This finding was matched with Comsa *et al.*[26]. According to other researchers[10,27], a significant difference in VEGF expression between IDC and other histological types was demonstrated. However, the number of our included non IDC histological types was too small to document such a conclusion.

Although, the majority of the studied cases were grade 3 (14/18) and grade 2 (44/80) carcinoma and were positive for VEGF, present study failed to prove any correlation between high tumor grade and VEGF overexpression. This finding is similar to finding reported by others[4,10]. Previous studies reported a significant correlation between tissue expression of VEGF and tumor grade of the breast tumors[7,27,31]. Previous studies reported an inverse correlation with tumor grade where the expression was higher in low grade tumors and reduced as the grade progress[14,19]. They explained that VEGF is important for early tumor but later on in advanced breast cancer the expression could be reduced due to expression of other angiogenic factors like platelet-derived endothelial growth factor and transforming growth factor.

A previous report showed that VEGF mRNA expression in estrogen-dependent breast cancer cell line MCF-7 is down-regulated by estrogen[34]. Similarly, others confirmed a significant relation between IHC expression of VEGF and negative hormonal receptor status[12,35] and concluded the poor prognosis of VEGF positive tumor. However, it could not confirm this relationship as no significant association between VEGF and hormonal status (ER and PR) was reported. Others agree with our result[4,34].

Based on the fact that VEGF assessment in the circulation might provide a noninvasive and repeatable method to get information about tumor vascularity and it may reflect the bulk of tumor-cell[23], the association between VEGF tissue expression by IHC and serum level by ELISA was analyzed. The VEGF protein is synthesized by breast carcinoma cells and they contribute considerably to circulating VEGF levels[11]. Thus, tissue expression was considered as the gold standard and serum level was the test. To our knowledge, there were only few reports found in the literature regarding this association[7,11,12,32].

Finally, we investigated whether there was a correlation between circulating VEGF and the IHC tissue expression; no significant relationships were found confirming results done by others[11,12,32]. In contrast, Ali *et al.*[7] demonstrated a positive correlation between tissue and serum VEGF expression in breast carcinoma cases and concluded that serum marker might be a biologically and clinically useful marker in diagnosing breast cancer and identifying high risk group[7].

CONCLUSION

In conclusion, VEGF is overexpressed in breast carcinomas compared to the benign tissue so its expression is considered to be an important indicator of the malignancy in breast tumors. Furthermore, tissue expression of VEGF can be used as a prognostic marker due to the significant association with the large tumor size, lymph node metastasis and positive Her2-neu status. In the contrary, no significant correlation was found between VEGF tissue expression and its serum level.

SIGNIFICANT STATEMENTS

Breast cancer is the most common female cancer both in the developed and less developed world. In Egypt, breast cancer ranked first among cancers in females. Angiogenesis is an essential step for breast cancer growth, progression and dissemination.

The objective of this study is evaluation of sensitive novel diagnostic and prognostic markers in serum of primary breast cancer patients who are likely to have metastatic cancer disease.

Determination of new effective low cost and noninvasive biomarkers may be more valuable for the early diagnosis, prognosis and staging of the disease and can support clinicians in their daily routine. However, analyses tools need to be standardized and simplified in order to be useful, reliable and widely available.

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