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Original Research Article

Vascular endothelial growth factor –A (VEGF-A) expression as a prognostic factor in breast cancer

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ABSTRACT

Objective: To assess the expression of VEGF-A in breast cancer patient and to find an association between VEGF- A overexpression and the clinicopathologic features.
Materials and Methods: The study was conducted from January 2010 through 2016. Formalin-fixed, paraffin-embedded blocks from 64 patients with breast cancer were included in this study. S treptavidin-biotin method was employed for immunohistochemical detection of VEGF.
Results: The detection rate of VEGF was 93.5%. There was a significant difference in the immunoexpression of VEGF A between the different histological types of carcinoma. However, no significant differen ces were noted among age groups, tumor sizes, perineural invasion and overall survival.
Conclusion: In our study, VEGF overexpression was positiv ely associated with only the histological type of breast cancer. Further studies involving patients with advanced diseases are required to establish an association between the VEGF-A over expression and survival outcomes and to use it as a prognostic biomarker.

1. Introduction

Breast cancer is the most common cancer and the leading cause of cancer related mortality in women.[1] Similar to other solid tumors, breast cancer also depends on tumor angiogenesis for the supply of nutrients and oxygen, thereby promoting the spread of cancer cells.[2] Tumor angiogenesis occurs predominantly during the developmental and progression stages of cancer and plays a vital role in metastasis. Evidences from literature has demonstrated significant correlation between the VEGF expression in tumor tissue and microvessel density (MVD) in many solid tumors.[3,4] In breast cancer, the MVD has been reported as an important risk factor for metastasis and is associated with poor prognosis.[5,6] Hence, there has been lot of interest in exploring the role of angiogenesis in the diagnostic and therapeutic management of breast cancer.

Tumor angiogenesis is mediated primarily by vascular endothelial growth factor A (VEGF-A).[7,8]

VEGF-A is a secreted ligand that has specific receptors (VEGF-R1 and -R2) and is involved in the process of endothelial cell growth, motility, and blood vessel permeability. The study by Hugo Arias-Pulido and colleagues demonstrated an increased risk of breast cancer death and recurrence in patients with high VEGF- A expression at baseline independent of clinico-pathological risk factors and treatment. They concluded VEGF-A expression levels at diagnosis to be an effective prognostic factor allowing individualization of therapy.[9]

Further, studies have reported significant correlation between VEGF-A concentration and MVD in patients with breast cancer.[10] The study by Xie at al investigating the relationship between MVD, expression of VEGF-A and micro-metastases in peripheral blood of patients with breast cancer has reported a positive correlation between VEGF-A expression and axillary lymph node metastasis.[11] As some

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of the breast cancers exhibit high angiogenic characteristics, several trials have evaluated the role of anti- angiogenic agents targeting VEGF-A as an therapeutic approach in breast cancer.[12–14]

As VEGF-A has been found to be an independent poor prognostic indicator for disease free survival and overall survival, this can be used as a biomarker to identify a subset of breast cancer at higher risk for development of recurrence and distant metastasis.[15] Hence this study was undertaken to study the clinic-pathological association of VEGF- A in relation to demographic parameters, stage, histopathology, IHC markers and survival parameters in Indian women with breast cancer.

2. Materials and Methods

2.1. Study design and population

This was an exploratory non randomized retro-prospective observational study conducted at the Apollo Hospital, Bangalore. Data were collected retrospectively from 64 consecutive patients who had a histopathological diagnosis of breast cancer during January-June 2010. The patients were followed up till June 2016.

Patients were included in the study if they had: i) histologically confirmed breast cancer and had received treatment at our institute ii) patients who had ER /PR /Her2neu /Ki67 markers evaluated. Further, the patients who did not complete treatment and those who did not accept the planned treatment were excluded from the study.

Early breast cancer and locally advanced breast cancer patients were followed after completion of primary treatment every 3 months for 3 years then once in 6 months for 2 years.

The study protocol was approved by the institutional review board while confirming to the standards of the Declaration of Helsinki and its subsequent revisions.

2.2. Pathology and immunohistochemistry

Sixty-four paraffin-embedded archival specimens of breast cancer were retrieved and assessed for the histological type (invasive ductal carcinoma or other types), grade as per the Scarff -Bloom-Richardson grading system (grade I well differentiated, grade II moderately differentiated, grade III poorly differentiated), lymphovascular invasion, margins (positive or negative for tumor cells), perineural invasion and the number of lymph nodes involved by tumor.

2.2.1. Immunohistochemistry

The slides previously immunohistochemically stained according to the manufacturer's guidelines (Dako, Glostrup, Denmark), were retrieved from the archives and reviewed by pathologists. To estimate the percentage of cells that stained positive for ER and PR by the Allred score, 100 tumor cells were counted, and the ratio of the number of stained cells to the total number of cells was calculated and reported as a percentage. The intensity of staining was graded as negative, weak, moderate, or strong. Both parameters (percentage and intensity) were considered in the Allred scoring system of ER and PR status. The tumor cells with $>20\%$ stained were considered as positive, those with 5% to 19% stained were considered as borderline, and those with $<5\%$ stained were designated negative; however, finally all the tumor cells with $>5\%$ stained were considered positive.

For the Her2neu overexpression, the data were classified from 0 to 3 based on the criteria provided by Dako (Glostrup); the scores 0 and 1+ were considered as negative, 2+ as borderline, and 3+ as positive.[16] All the cases with a 2+ score were sent for the FISH test, and cases with a positive FISH were counted as HER2neu positive.

The proliferative activity was determined by immu nostaining for the Ki-67 antibody (Dako, Glostrup, Denmark). The fraction of proliferating cells was based on a count of at least 500 tumor cells. The Ki-67 values were expressed as the percentage of positive cells in each case. The Ki-67 immunohistochemically stained slides for Ki-67 marker were divided into 2 groups: the cells that stained over 15% were considered as high Ki-67 expression and $<=$ 15% stained cells were considered as low Ki-67 expression.[17]

For VEGF-A, the immunohistochemical staining was performed by the streptavidine -biotin method. Sections with a 5-μm thickness were deparaffinized and incubated with 0.3% hydrogen peroxide for 20 min to block the endogenous peroxidase activity. After being washed with Phosphate-buffered saline (PBS), sections were incubated with anti-VEGF-A for 1 hour at room temperature. Followed by two washes with PBS, sections were incubated with biotinylated secondary antibody for 10 minutes at room temperature, followed by washes and were treated with streptavidine peroxidase reagent for 10 min at room temperature. Sections were washed again twice and were incubated with diaminobenzidine solution for 5 min. Finally, the slides were counterstained with Mayers' hematoxylin and mounted. VEGF-A expression data were classified from 0 to 3 based on the criteria system described by Raica and colleagues.[16]

2.3. Statistical analysis

Cross tables were made to know the frequency distribution of the variables, cross tables were tested by using Chi-square test where expected frequency was more than 5, and otherwise fisher exact test was used with 95% confidence level. Survival curves were constructed using the Kaplan-Meier product-limit method and statistical significance was assessed using the log-rank test. R Software was used to analyze data**.** A p value of < 0.05 was considered as significant.

3. Results

3.1. Clinicopathologic characteristics

A total of 64 patients with a median age of 52.50 years were included in the study. About 76.6% (n=49) had attained menopause and remaining 23.4% (n=15) were premenopausal status at the time of presentation. About 6.3% of the study population had positive family history of breast cancer. The clinicopathologic features of the study population are outlined in Table 1.

3.2. Immunohistochemical characteristics

Among the study subjects, 3 patients (4.7%) were negative for VEGF A score while 17 patients (26.6%) had 1+ VEGF-A score, 28 (43.8%) demonstrated 2+ VEGF-A score and 16 patients (25%) had 3+ VEGF-A score.

3.3. Association of VEGF-A with histopathological features

In our study the VEGF-A expression was significantly higher in invasive ductal carcinoma (IDC) compared to the other histological types, suggestive of a positive association between VEGF-A expression and the histological type of breast cancer (Table 2).

There was no significant association between the VEGF-A expression and other clinicopathological features including age (p= 0.063), menopause (0.238), tumor stage (0.132), tumor size (p=0.899), lymph nodes (p=0.195), lymphovascular invasion (p=0.383), perineural invasion (p=0.380) and estrogen receptor status (p=0.784). There was no significant association between VEGF-A expression and treatment modalities including chemotherapy and hormonal therapy.

In terms of overall survival, 2 patients each died in VEGF-A 1+, 2+ and 3+ expression groups respectively. Further there was no association of VEGF-A expression with survival (p= 0.871), Figure 1.

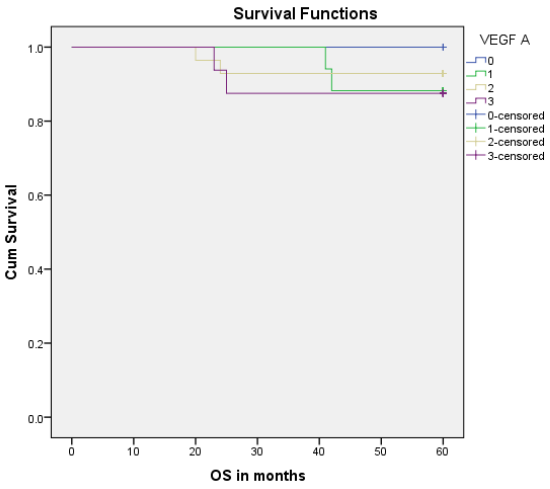

Fig. 1: Kaplan–Meier survival curves showing association of VEGF-A with overall survival.

4. Discussion

VEGF is a potent angiogenic growth factor that acts through receptor mediated pathways and is a potential target for antiangiogenic therapy.[17,18] In this study, we evaluated the association of VEGF-A expression with clinicopathological parameters and the survival outcome in 64 breast cancer patients at Apollo Hospital, Bangalore.

In the current study, positive over-expression of VEGF-A was noted in 95.3% of all the cases. This is higher than that reported previously including the studies by Cimpean et al. (87.3%) and Nakamura et al. (83.7%).[16,19] A variability of positive cases may be due to sample size and different clone of VEGF-A. In our study, there was no significant association of VEGF-A expression with that of age and menopausal status. The results of our study are similar to that reported by Cimpean and colleagues, where in they reported non-significant correlation (p = 0.1438) between age and VEGF expression.[16]

The study by Jacobs EJ et al. demonstrated an association between increased VEGF expression and the increased risk of invasive breast cancer.[20] Similarly, Cimpean et al. reported a significant association of VEGF expression with that of ductal invasive type of breast cancer.[16] Further the recent study by Almumen M et al. reported significant difference in the association of VEGF with ductal carcinomas than that of infiltrating lobular carcinomas (65.91% versus 25%, $p < 0.05$).[21] Based on these lines, our study also demonstrated a significant association of VEGF-A expression with th at of the histology of the breast cancer.

Though there are studies reporting strong association of VEGF-A with tumor size,[15,22] in our study there was no significant association of VEGF-A expression and tumor size. Similar to the results of our study, Almumen M et al[21] and Srabovic N et al.[2] have reported no significant association of VEGF-A expression with the tumor size.

Numerous studies have reported statistically significant association of VEGF-A expression with overall survival.[15,23–25] However in our study there was no significant association between the overall survival and the VEGF-A overexpression.

Our study had few limitations including the smaller sample size used for analysis of VEGF-A with clinicopathological markers, short follow up duration and the smaller number of metastatic patients included in the study.

5. Conclusion

The heterogeneous nature of breast cancer with several different subtypes with different molecular profiles,

Table 1: Clinicopathologic characteristics in study population

	Median	Patients (%) (n=64)
Age, years 52.50		
<40		7 (10.9%)
41 – 59		38 (59.37)
> 60		19 (29.68)
Presenting Complaints		
Lump		62 (95.3)
Cough		2(4.7)
Menopausal Status		
Pre-menopausal		15 (23.4)
Post-menopausal		49 (76.6)
Tumor Stage		
I A		2 (3.1)
II A		20 (31.3)
II B		14 (21.9)
III A		15 (23.4)
III B		6 (9.3)
III C		3 (4.7)
IV		4 (6.25)
Histological Type		
IDC		60 (94)
Medullary		3 (5)
Secretory carcinoma		1(1)
Lymphovascular Invasion		
Negative		31 (48.5)
Positive		33 (51.5)
Perineural Invasion		
Negative		45 (70.3)
Positive		19 (29.7)
ER Status		
Negative		28 (43.8)
Positive		36 (56.2)
Adjuvant Chemotherapy		
Yes		49 (76.6)
No		15 (23.4)
Radiotherapy		
Yes		40 (62.5)
No		24 (37.5)
Adjuvant Hormonal Therapy		
Yes		37 (57.8)
No		27 (42.2)

Table 2: Association of VEGF-A with histology

VEGF A	Medullary		IDC		Secretory carcinoma		Total	P-Value
	n	%	n	%	n	%	n	
0	1	33.3	2	3.3		0.0	3	
1		0.0	17	28.3		0.0	17	
2		0.0	28	46.7		0.0	28	0.041
3	2	66.7	13	21.7	1	100.0	16	
Total	**3**	**100.0**	**60**	**100.0**	**1**	**100.0**	**64**	

biological behavior, and risk profiles poses a challenge for therapeutic decision making process. From the data currently available on the role of VEGF-A expression in breast cancer, it may be concluded that results are contradictory and still inconclusive. In our study, VEGF-A expression as an independent prognostic factor in breast cancer was associated only with histology.

6. Source of funding

None.

7. Conflict of interest

None.

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