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Study of expression and correlation of vascular endothelial growth factor with grading and staging of breast carcinoma

Authors
Dr Nataraju G, Dr S.A.K Adil, Dr Jayani.C, Dr Bharathi M
Department of Pathology, Mysore Medical College and Research Institute, Mysuru, Karnataka, India
Corresponding Author
Dr S.A.K. Adil
Department of Pathology, Mysore Medical College and Research Institute, Mysuru, Karnataka, India
Phone number– 9019691259, Email: *Salla291@Yahoo.co.in*

Abstract
Breast cancer is the most frequent malignancy in women all over the world, resulting in death in one forth of the cases. To date, many efforts are being performed to identify prognostic factors and their relevant therapeutic agents. Tumor induced angiogenesis is essential for tumor growth, invasiveness and evolution of breast cancer. It has been proven that vascular endothelial growth factor (VEGF) are essential for neovascularization and progression of breast cancer. The objective of this study is to study the expression of VEGF in breast carcinomas and to correlate the expression of VEGF with histological grade and stage of breast carcinoma. In this study, 30 cases of histological proven invasive breast carcinoma- no special type were analyzed for vascular endothelial growth factor expression by immunohistochemical staining and its correlation with the grading and staging of breast carcinoma. Vascular endothelial growth factor expressed in 93.33% of the breast carcinomas. According to this study there is a significant correlation between tumor size and VEGF expression (P<0.05) while there was no significant correlation between VEGF expression and grading and lymph node involvement of breast carcinoma. expression of VEGF can be used as a prognostic marker due to the significant correlation with the tumor size.
Keywords: *Angiogenesis, breast cancer, TNM staging, Scar -Bloom-Richardson grading, VEGF*.

Introduction

Breast carcinoma ranks first among the malignant tumours affecting females in many parts of the world [(1)].

A considerable body of research spanning almost three decades has documented that tumor growth and metastasis require persistent new blood vessel growth the absence of access to an adequate vasculature, tumor cells become necrotic and/or apoptotic restraining the increase in tumor volume that should result from continuous cell proliferation, the hallmark of cancer[(2)].

Over time, cancer cells produce and release angiogenic growth factors at levels that overwhelm the suppressive effects of endogenous inhibitors [(3)].

Vascular endothelial growth factor (VEGF) is one of the most commonly studied vascular growth factors in human tumors. Increased expression has been suggested to be involved in tumorigenesis,

metastasis, and the production of malignant effusion caused by enhancement of vascular permeability and/or angiogenesis in primary breast carcinoma [4].

Several anti-angiogenic drugs have been developed and are being tested in clinical trials or used as standard treatment for different types of cancer. The increasing number of expensive drugs in cancer treatment emphasizes the need for predictive markers to select the patients who are most likely to benefit from the treatment. Because VEGF plays a key role in angiogenesis, reliable measurements of the ligands and the receptors are of crucial importance from both a clinical and a biological point of view [5].

Materials and Methods

This study was carried out in the department of pathology, Mysore Medical College & Research Institute on 30 cases of breast carcinoma. The modified radical mastectomy specimens of breast carcinomas were received in 10% formalin. They were grossed and macroscopic features were noted. The tissues were processed. Paraffin sections were cut into 3-5 µm in thickness and stained with hematoxylin and eosin.

Pathologic staging depending on tumor size and lymph node status was done according to the TNM Staging. Histologically, tumors were graded as I–III (Scar -Bloom-Richardson grading) based on tubule formation, nuclear grade and mitotic count.

Additional slides from the primary tumors were processed for immunohistochemical identification of VEGF expression The technique for IHC included antigen retrieval in EDTA buffer in a pressure cooker, blocking endogenous peroxidase with 3% hydrogen peroxide, incubating with primary rabbit polyclonal antibody (Bio Genex ,Netherlands), linking with secondary antibody, developing chromogen with deaminobenzidine (DAB) and counterstaining with haematoxylin .

The immunostained slides were examined for cytoplasmic staining of VEGF.

VEGF Scoring

We applied for all cases scoring system described by Raica M *et al.* for VEGF expression in intestinal type of gastric carcinoma[6](Table 1).

Statistical Analysis

Statistical analysis was performed with SPSS13.0 software. For normally distributed variables Pearson's Correlation test, $p<0.05$ is being considered as significant. For variables not normally distributed Spearson's correlation test, $p<0.05$ is being considered significant.

Results

This study was performed on 30 cases of invasive carcinoma- no special type. The mean age of breast cancer patients was 50 years at the time of their cancer diagnosed. 20 % of tumors were classified as grade I, 50% as Grade II and 30% grade III.

VEGF was detected by IHC staining in 28 cases (93.33%) in the cytoplasm of tumor cells with granular pattern. Using VEGF scoring described above, we found 20 cases with +3 pattern, six cases with +2, and 2 cases noted with +1. The intensity of staining was heterogeneous but most of the cases showed moderate to intense expression of VEGF. By statistical analysis we found no significant correlation ($p = 0.06$) between VEGF expression and the grade of breast cancer.

We also evaluated the tumor lesions according to the TNM staging system. Taking into account the tumor dimensions, two cases were staged as T1c, 19 as T2, six as T3, one as T4a and two as T4b. Based on the number of lymph nodes involved, eight cases were pN0, 12 cases were pN1a, four were pN2a, three were pN3a and three cases of pNx. By statistical analysis we found a significant correlation ($p=0.027$) between expression of VEGF and the size of the tumor while there was no significant correlation between VEGF expression and the lymph node metastasis ($p=0.16$)

Table 1 VEGF Scoring

Score	Staining	Positive tumor cells (%)	Intensity
0	No	<1%	_
1	+	1-25%	Weak
2	++	26–50%	Moderate
3	+++	>50%	Strong

Figure 1-Positive staining for VEGF noted with +1

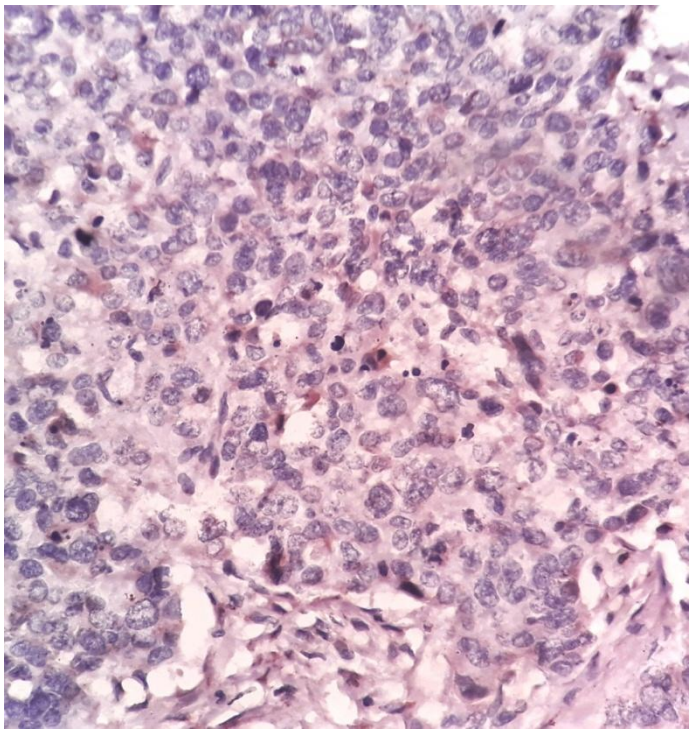


Figure 2-Positive staining for VEGF noted with +2

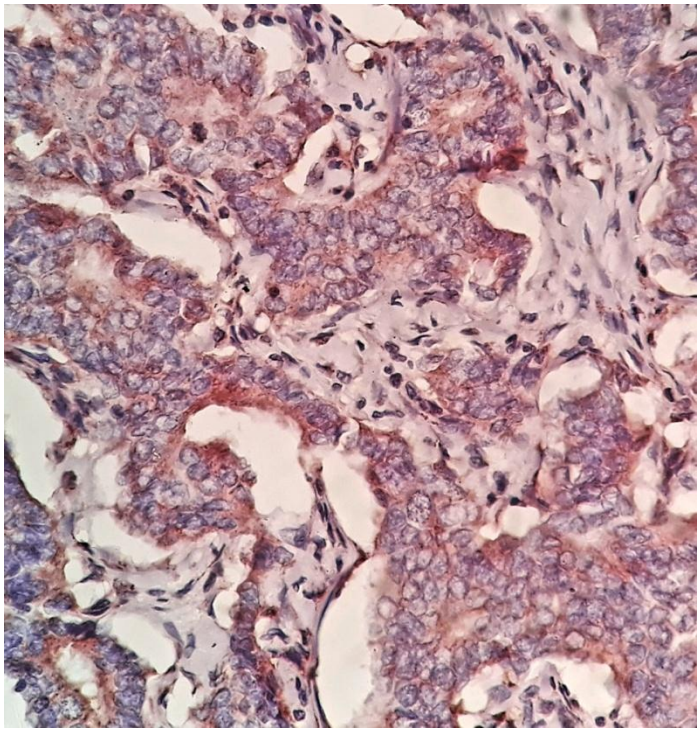
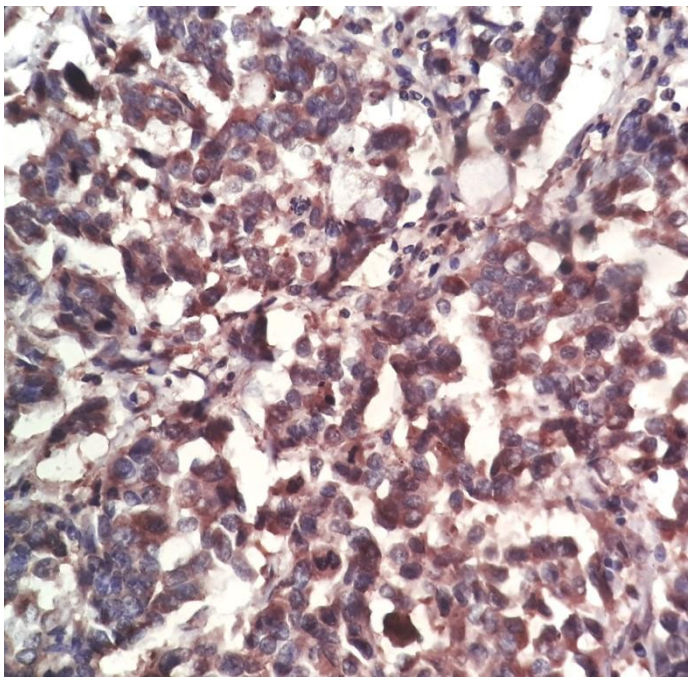


Figure 3-Positive staining for VEGF noted with +3



Discussion

Breast carcinoma is a heterogeneous disease with variations in clinical behaviour such as response to treatment, metastatic potential, and survival, even among patients with the same clinical or pathologic stage. Therefore, it would be of utmost interest to detect subgroups of patients at high risk for relapse to design and deliver an optimal treatment[4].Growth, invasion, and metastasis of many cancers depend on angiogenesis[7].

Virtually all tumors begin their existence as small clusters of a vascular cells that cannot grow beyond 2-3 millimeter in diameter until a new blood supply can be recruited. The period between the prevascular state (carcinoma in situ) and the vascular state (invasive carcinoma) may last for decades[3].

Among the most important of these is vascular endothelial growth factor (VEGF). This growth factor may be produced in response to environment stimuli, mainly hypoxia, and to stimulation by certain cytokines and estradiol[8].

On the other hand, therapeutic blockade of VEGF has been shown to inhibit primary and metastatic tumor growth in animal models. Art1 Anti-VEGF therapy with bevacizumab, a humanized

monoclonal antibody against VEGF, shows an improvement in progression-free survival in combination with chemotherapy for women with metastatic breast cancer [9].

Therefore, VEGF could be an important marker of angio- genic activity for prognostic purposes as well as for target- ing inhibition of angiogenesis as a novel therapeutic strategy against cancer [7].

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.

This study analyzed VEGF expression by IHC in invasive carcinoma of breast cases and the correlation of VEGF expression with the grading and staging of breast carcinoma.

In our study we found 93.33% of cases showing positivity for VEGF expression. Similar results were seen in some of the previous studies [10,11,12,13]. High number of positive cases for VEGF sustains the concept that this growth factor is involved in the development of breast tumors by different mechanism [10].

In our study we demonstrated a weak correlation between the histological grade and the VEGF expression which was not significant (p-0.06). This finding was in accordance with the results of others similar [8,10,11,14]. On the other hand Linderholm B et al.[4] reported a significant correlation between VEGF expression and grading of breast carcinoma while Ghasemi M et al.[13] showed inverse correlation.

In our study we demonstrated, VEGF tissue expression was significantly associated with large tumor size (p-0.027). Similar results were observed in other previous studies[4,14,15,16], Thus, confirming the dependence of tumor expansion on angiogenesis[14]. In contrast, no significant correlation was found in previous studies [8,10,11]. Comsa et al. [12] reported an inverse correlation between VEGF expression and tumor size.

We demonstrated in our study that lymph node involvement had no significant correlation with VEGF expression similar to the observations of Almumen, M [8] several other studies demonstrated significant correlation between VEGF expression and lymph node involvement[10,11,14].

Conclusion

In conclusion, VEGF expression in breast carcinomas can be considered as an important indicator of the malignancy in breast tumors. Furthermore, expression of VEGF can be used as a prognostic marker due to the significant correlation with the tumor size. However, the relationship between VEGF expression and histological grading and lymph node involvement remains unclear.

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