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ORIGINAL ARTICLE

Immunohistochemical Expression of Vascular Endothelial Growth Factor in Invasive Breast Carcinoma and Its Correlation with Clinicopathological Parameters

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Abstract

Background: Vascular endothelial growth factor (VEGF) is a critical mediator of tumor angiogenesis and has been implicated in the growth, invasion, and metastatic spread of invasive breast cancer. In this work, VEGF expression in invasive breast cancer was assessed using immunohistochemistry (IHC) and associated with known clinicopathological factors. **Methods:** The Department of Pathology at Government Kilpauk Medical College in Chennai carried out this cross-sectional study over the course of eighteen months, from January 2023 to June 2024. Included all 43 histologically verified cases of invasive breast cancer from modified radical mastectomy specimens that had not received any prior radiation or chemotherapy by consecutive sampling. VEGF immunopositivity (cytoplasmic, brown staining) was measured semi-quantitatively at 40× magnification and linked with age, tumor size, histological grade, lymph node status, and co-expression of ER, PR, HER2/neu, and Ki-67 using the chi-square test (significance at $p<0.05$). **Results:** VEGF positive was reported in 29/43 instances (67.44%; $p<0.001$). Positivity increased with advancing age (100% in 61–70 years) and bigger tumor size (100% in tumors >5 cm; $p<0.001$), and was substantially linked with lymph node status and HER2/neu, ER, and PR co-expression (all $p<0.001$). VEGF expression and histological grade did not significantly correlate ($p>0.05$). **Conclusion:** VEGF is frequently expressed in invasive breast cancer and correlates significantly with tumor size and lymph node status – recognized indicators of aggressive disease — indicating its potential relevance as a prognostic biomarker and a target for anti-angiogenic therapy.

Keywords: Vascular endothelial growth factor, Invasive breast carcinoma, Immunohistochemistry, Angiogenesis, Prognostic marker

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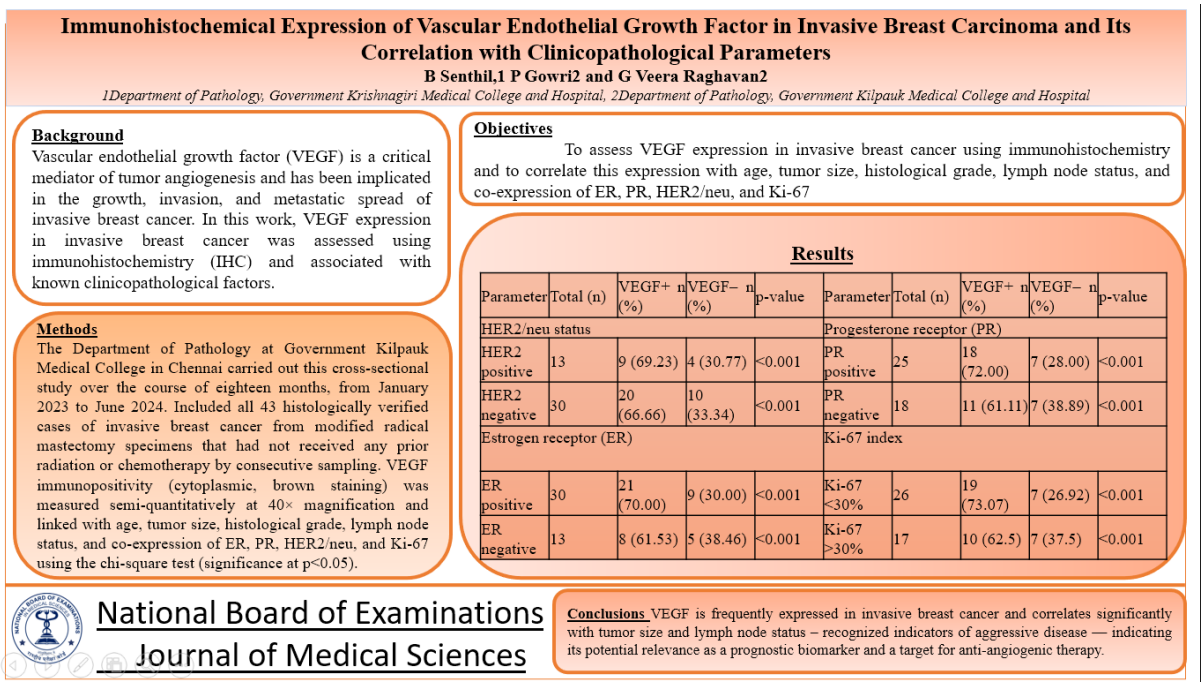
Graphical Abstract



Introduction

Breast carcinoma is the most frequent malignancy among women globally and accounts for nearly one-fourth of all female cancers [1]. Hematogenous metastasis and tumor growth beyond a few millimeters depend on tumor angiogenesis, which is the creation of new blood vessels from pre-existing vasculature [2]. This process is primarily driven by vascular endothelial growth factor (VEGF), which is found on chromosome 6p12. VEGF enhances vascular permeability, stimulates endothelial proliferation, and accelerates extracellular matrix disintegration, all of which enable tumor invasion [3].

An increasing body of evidence links VEGF expression to conventional prognostic factors in breast cancer, including tumor size, histological grade, lymph node status, and hormone receptor (ER/PR) and HER2/neu status, however findings across studies remain inconsistent. Characterizing VEGF expression and its clinicopathological correlates in a local population is important for both diagnosis and treatment because of its prominent role in the angiogenic cascade and its development as a target for anti-angiogenic therapy.

The purpose of this study was to assess VEGF expression in invasive breast cancer using immunohistochemistry and to correlate this expression with age, tumor size, histological grade, lymph node status, and co-expression of ER, PR, HER2/neu, and Ki-67.

Materials and Methods

Study design and setting

This cross-sectional study was conducted in the Department of Pathology, Government Kilpauk Medical College, Chennai, over a period of 18 months (January 2023 to June 2024).

Study population

Histologically confirmed invasive breast carcinoma (NOS) from modified radical mastectomy (MRM) specimens. Cases that had received chemotherapy or radiotherapy prior to surgery, excision/tru-cut biopsies, and poorly fixed specimens were excluded.

Sample size and Sampling technique

Universal sampling was employed. All 43 cases of histologically confirmed invasive breast carcinoma that fulfilled the predefined inclusion and exclusion criteria during the study period (January 2023 to June 2024) were consecutive included in the study.

Methods

Specimens were fixed in 10% formalin, processed, and paraffin-embedded; 4 µm sections were mounted on silanized slides and stained with haematoxylin and eosin. Clinicopathological parameters — age, tumor size, focality, histological type and grade, lymph node status, lymphovascular and perineural invasion — were recorded, along with IHC status for ER, PR, HER2/neu, and Ki-67. VEGF immunohistochemistry was performed on representative blocks; immunopositivity was cytoplasmic and brown, assessed as the percentage of positive cells among 100 malignant cells at 40× magnification, and scored using a standardized 0–4+ scale. [3] After antigen retrieval and incubation with the primary antibody, immunoreactivity was visualized using a DAB detection system. Appropriate positive and negative controls were included in each staining run to ensure staining quality.

Statistical analysis

Data were entered into Microsoft Excel and analyzed using appropriate statistical software. Associations between VEGF expression and clinicopathological variables were primarily assessed using the Chi-square test. The assumptions for the Chi-square test were verified prior to analysis. Where expected cell counts were less than five, Fisher's exact test was used as appropriate**.** A *p*-value of <0.05 was considered statistically significant.

Results

Of the 43 cases of invasive breast carcinoma studied, VEGF positive expression was observed in 29 cases (67.44%) and negative expression in 14 cases (32.56%) (Table 1).

Table 1. Overall VEGF expression in invasive breast carcinoma (n=43)

Total cases (n)	VEGF positive n (%)	VEGF negative n (%)
43	29 (67.44%)	14 (32.56%)

VEGF positive exhibited a growing tendency with advancing age, from 66.66% in the 31–40-year group to 100% in the 61–70-year group. Positivity also increased with tumor size, reaching 100% in tumors larger than 5 cm (T3), compared with 62.85–66.67% in smaller tumors (all $p<0.001$). No statistically significant correlation was discovered between VEGF expression and histological grade ($p>0.05$), but Grade I tumors showed the highest positive (100%). VEGF expression was

substantially linked with lymph node status, HER2/neu status, ER status, PR status, and Ki-67 index (all p<0.001), with a consistently greater proportion of VEGF positivity across both positive and negative subgroups of each marker (Table 2).

Table 2. Correlation of VEGF expression with clinicopathological parameters

Parameter	Total (n)	VEGF+ n (%)	VEGF– n (%)	p-value
Age (years)				
31–40	3	2 (66.66)	1 (33.34)	<0.05*
41–50	14	8 (57.14)	6 (42.86)	
51–60	22	15 (68.18)	7 (31.82)	
61–70	4	4 (100.00)	0 (0.00)	
Tumor size				
T1 (≤2 cm)	3	2 (66.67)	1 (33.33)	<0.001*
T2 (2–5 cm)	35	22 (62.85)	13 (37.15)	
T3 (>5 cm)	5	5 (100.00)	0 (0.00)	
Tumor grade				
Grade I	3	3 (100.00)	0 (0.00)	>0.05*
Grade II	29	19 (65.52)	10 (34.48)	
Grade III	11	7 (63.64)	4 (36.36)	
Lymph node status				
LN positive	24	16 (66.66)	8 (33.33)	<0.001*
LN negative	19	13 (68.42)	6 (31.57)	
HER2/neu status				
HER2 positive	13	9 (69.23)	4 (30.77)	<0.001*
HER2 negative	30	20 (66.66)	10 (33.34)	
Estrogen receptor (ER)				
ER positive	30	21 (70.00)	9 (30.00)	<0.001*
ER negative	13	8 (61.53)	5 (38.46)	
Progesterone receptor (PR)				
PR positive	25	18 (72.00)	7 (28.00)	<0.001*
PR negative	18	11 (61.11)	7 (38.89)	
Ki-67 index				
Ki-67 <30%	26	19 (73.07)	7 (26.92)	<0.001*
Ki-67 >30%	17	10 (62.5)	7 (37.5)	

*p-value<0.05-Statistically significant

Discussion

Tumor angiogenesis, progression, and metastasis are all significantly influenced by VEGF, and its expression has been connected to known prognostic factors in breast cancer, including tumor size, tumor grade, lymph node status, and hormone receptor and HER2 status. The conclusion that VEGF expression is common in invasive breast cancer is supported by the fact that 67.44% of patients in the current investigation had VEGF positivity, which is similar to the 59.62% positivity reported by Almumen et al. [3].

In contrast to the results of A. Pa et al., who found no correlation between chronological age and VEGF levels, VEGF positive rose with age in this cohort, reaching 100% in the 61–70-year group, suggesting a potential age-related trend in angiogenic activity. A substantial positive connection was also seen between tumor size and VEGF expression, with all tumors larger than 5 cm showing positivity — a trend largely comparable with Almumen et al., who also reported the highest VEGF positivity in the largest tumor-size category [3]. This lends credence to the idea that tumor growth is accompanied by an increase in angiogenic drive.

In contrast, no significant association was found between VEGF expression and histological grade in this study, in agreement with the view that other biological factors may more strongly influence VEGF expression in invasive ductal carcinoma than grade alone; this differs from Shera et al., who reported increasing VEGF levels with higher tumor grade and stage [7].

VEGF expression was substantially related with lymph node status, being high in both LN-positive (66.66%) and LN-negative (68.42%) groups, demonstrating that VEGF elevation is not confined to node-positive illness alone. Similarly, high VEGF positivity was observed across both HER2/neu-positive and -negative groups, comparable to the findings of Jannat Shakila et al. and Almumen et al. [3,8]. Co-expression analysis with hormone receptors showed higher VEGF positivity in both ER-positive (70%) and PR-positive (72%) cases, in keeping with the findings of Jannat Shakila et al. and Konecny et al., although Almumen et al. reported the reverse trend (higher positivity in ER-negative tumors), indicating that this relationship may vary across populations [3,8,9]. VEGF positivity was also higher in tumors with lower Ki-67 proliferation index (<30%), suggesting VEGF up-regulation may occur independent of proliferative activity in this group.

Although statistically significant associations were observed between VEGF expression and certain clinicopathological parameters, including lymph node status and HER2/neu expression, the absolute differences in VEGF positivity between some comparison groups were relatively small. Therefore, these findings should be interpreted with caution, as statistical significance does not necessarily equate to clinical significance. Larger studies with adequate statistical power and longitudinal follow-up are required to determine the true clinical relevance and prognostic impact of these associations.

Collectively, these data reaffirm VEGF as a marker intimately connected to tumor volume and nodal involvement — both established indications of aggressive illness — but its relationship with grade and proliferation markers is more variable among studies.

Conclusion

VEGF expression was observed in a majority of invasive breast carcinoma cases and demonstrated significant associations with tumor size, lymph node status, and selected clinicopathological parameters. These findings support the potential role of VEGF as a biomarker of tumor angiogenesis and disease aggressiveness in invasive breast carcinoma. Further large-scale prospective studies are warranted to validate these observations and to better define the prognostic and therapeutic relevance of VEGF expression in routine clinical practice.

Limitations

This study has certain limitations that should be considered while interpreting the findings. First, it was conducted at a single tertiary care center with a relatively small sample size, which may limit the generalizability of the results to other populations. Second, the cross-sectional study design precludes assessment of temporal relationships and does not permit evaluation of the prognostic significance of VEGF expression with respect to disease recurrence, disease-free survival, or overall survival. Third, owing to the limited sample size, multivariable analysis was not performed; therefore, the independent prognostic value of VEGF after adjusting for potential confounding clinicopathological variables could not be established. Finally, VEGF immunohistochemical staining was not evaluated by a single pathologist, and inter-observer variability was not assessed, which may have introduced observer-related bias despite the use of standardized scoring criteria. Future multicentric studies with larger cohorts, longitudinal follow-up, and multivariable analyses are warranted to validate these findings.

Authors' Contributions

BS has contributed to the conceptualization and definition of the intellectual content of the manuscript, design of the study and Manuscript preparation. PG contributed to the literature search, manuscript editing, and manuscript review. GVR contributed towards data acquisition Statistical analysis, Manuscript review and editing. BS will act as the corresponding author of the manuscript.

Data availability statement

The datasets generated and analysed in this study are available from the corresponding author on reasonable request. They are not publicly shared because they contain sensitive information that could indirectly identify participants.

Ethical Approval

This study has been approved by the Institution Ethics Committee Ref.No: ECR/1385/Inst/TN/2020

Informed Consent

Written informed consent was obtained from all participants after explaining the study procedures, potential risks and benefits. Consent covered both participation and publication of anonymised findings, with assurance of confidentiality and data privacy.

Conflicts of interest

The authors declare that they do not have conflict of interest.

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References

1. Singhal H, Gohel MS, Kaur K, Thomson S. Breast cancer evaluation. eMedicine. 2008:2–10.
2. Folkman J. The role of angiogenesis in tumor growth. Semin Cancer Biol. 1992;3(2):65-71.
3. Almumen M. Immunohistochemical VEGF levels in relation to other pathological parameters of breast carcinoma. J Cancer Ther. 2015;6(9):811–20. doi:10.4236/jct.2015.69089.
4. Nuka S, Devarakonda KK. Immunohistochemical VEGF levels and its relationship with tumor grade in breast carcinomas. J Cancer Dis. 2023.
5. Ribatti D, Nico B, Ruggieri S, Tamma R, Simone G, Mangia A. Angiogenesis and antiangiogenesis in triple-negative breast cancer. Transl Oncol. 2016;9(5):453–7. doi:10.1016/j.tranon.2016.07.002.
6. Pa A, et al. VEGF-A as individual prognostic factor in invasive breast carcinoma. Rom J Morphol Embryol. 2008;49(3):303–8.
7. Shera S, et al. VEGF levels in cancer of breast. Int J Adv Res. 2019;7(10):897–901. doi:10.21474/ijar01/9905.
8. Jannat S, Ahmed M, Syeed S, Khatun H, Afroze N. VEGF levels in breast carcinoma and its link with levels of estrogen receptors, progesterone receptors and HER-2/neu and molecular subtypes. Ann Int Med Dent Res. 2023;9(3):65–73. doi:10.53339/aimdr.2023.9.3.9.
9. Konecny GE, Meng YG, Untch M, Wang HJ, Bauerfeind I, Epstein M, et al. Association between HER-2/neu and vascular endothelial growth factor expression predicts clinical outcome in primary breast cancer patients. Clin Cancer Res. 2004;10(5):1706–16. doi:10.1158/1078-0432.ccr-0951-3.