Vascular Endothelial Growth Factor Expression in Breast Carcinoma and its Association with Expression of Estrogen Receptors, Progesterone Receptors and HER-2/neu and Molecular Subtypes

Shakila Jannat[1]*, Moushumi Ahmed[2], Sifat Syeed[3], Halima Khatun[4], Nazma Afroze[5]

[1]Assistant Professor, Department of Pathology, Khwaja Yunus Ali Medical College and Hospital, Sirajganj, Bangladesh.
Email: sjannat112@gmail.com
Orcid ID: 0000-0001-8239-4135
[2]Professor, Department of Pathology, BIRDEM General Hospital, Dhaka, Bangladesh
Email: mousumi7406@gmail.com
Orcid ID: 0009-0006-3089-1467
[3]Assistant Professor, Department of Pathology, Ad-Din Women's Medical College, Dhaka, Bangladesh.
Email: syeedsifat@gmail.com
Orcid ID: 0009-0006-0554-9301
[4]Assistant Professor, Department of Pathology, Khwaja Yunus Ali Medical College and Hospital, Sirajganj, Bangladesh.
Email: halimammc43@gmail.com
Orcid ID: 0009-0009-2893-0481
[5]Professor and Head, Department of Pathology, BIRDEM General Hospital, Dhaka, Bangladesh,
Email: afrozenazma@yahoo.com
Orcid Id: 0009-0002-2258-9111

*Corresponding author

Abstract

Background: The management of breast carcinoma depends on several molecular markers and tumor stages. In the last decades, estrogen receptors (ER), progesterone receptors (PR), and HER-2/neu have shown good therapeutic responses. Among other molecular markers, vascular endothelial growth factor (VEGF) is becoming more widely used as a prognostic indicator in patients with breast carcinoma. Anti-VEGF therapy already has been proven as an effective chemotherapeutic agent in some other carcinomas. The study aimed to find out the immunohistochemical expression of Vascular Endothelial Growth Factor (VEGF) in breast carcinoma and its possible correlation with the expression of ER, PR, and HER-2/neu and molecular subtypes to evaluate its prognostic value. **Material & Methods:** This study was conducted in the Department of Pathology, BIRDEM General Hospital, Dhaka, from March 2018 to January 2020. In this study, 45 diagnosed cases of breast carcinoma were enrolled. Slides of all cases were stained with ER, PR, HER-2/neu, and VEGF antibodies following the avidin-biotin-peroxidase staining method. Results: Among 45 cases, 60% showed positive immunohistochemical expression of VEGF. Most of these cases (71.1%) were ER/PR positive. VEGF did not show a significant association with other molecular markers or molecular subtypes. **Conclusion:** Although, the potential prognostic value of VEGF has not been confirmed. Based on the findings of the current study, it can be assumed that VEGF plays an important role in the pathogenesis of breast cancer. So, it may serve as a useful biomarker for immuno-targeting therapy in patients with breast cancer.

INTRODUCTION

Worldwide, there were 2.30 million women diagnosed with breast cancer and 685000 deaths were due to breast carcinoma in 2020. After 1980, breast cancer-related mortality declined gradually due to advanced diagnostic and therapeutic methods in developed countries. But, this cancer-related mortality remains very high in developing countries because of late diagnosis and delay in treatment.[1] Management of breast carcinoma depends mainly on the expression of some molecular markers like ER, PR, and HER-2/neu. These have been proven to have a great role in the

treatment and assessing the prognosis of breast carcinoma. Chemotherapy after surgery or neoadjuvant chemotherapy in patients with breast carcinoma is treated with target therapy against ER, PR expression, and HER-2/ neu over-expression by hormone therapy (Tamoxifen, Aromatase inhibitors such as letrozole, anastrozole) and anti-HER-2 antibody (Trastuzumab, pertuzumab, lapatinib) respectively. These have been proven to prevent the relapse and progression of the tumor.[2,3] Angiogenesis, the development, and formation of new blood vessels, is important in the development and progression of neoplasia. It is now believed that the sequential interaction between cancer cells and the newly formed blood vessels is a crucial step in the spread of cancer. Tumor cells with fast growth are believed to rely on continuous blood vessels for expansion. Vascularization is likewise considered to permit the shedding of cells from the primary tumor to distant metastasis. The regulation of new blood vessel growth is influenced by angiogenic and anti-angiogenic factors that are produced by tumor cells, inflammatory cells, and stromal cells. One of the key factors involved in this process is VEGF, which is the most powerful stimulant of blood vessel formation and plays a central role in promoting angiogenesis.[4,5,6,7] VEGF also plays an integral part in tumor growth by inducing the anti-apoptotic factors (Bcl-2 and surviving), which prevent apoptosis of newly formed vascular endothelial cells as well as tumor cells.[8] The expression of VEGF is being studied in many carcinomas and target therapy against VEGF has been studied for a long time. In 2004, for the treatment of metastatic colorectal carcinoma, renal cell carcinoma, and glioblastoma, Bevacizumab (anti-VEGF

therapy) gained approval from FDA. Anti-VEGF therapy improves the relapse-free survival period for these carcinomas. Several studies aimed at inhibiting the function of VEGF in breast carcinoma are currently under investigation. When combined with other chemotherapeutic agents, they show excellent responses.[9,10] The primary aim of this study is to determine the level of expression of VEGF in primary breast carcinoma and its correlation with other molecular expressions (ER, PR, and HER-2/neu). This study may help to determine the importance of anti-VEGF therapy for breast cancer as well as its prognostic value.

MATERIAL AND METHODS

The study material was represented by paraffin blocks of 45 histologically diagnosed cases of breast carcinoma collected from the Department of Pathology, BIRDEM General Hospital, Dhaka from March 2018 to January 2020. A re-evaluation of the routinely processed H&E-stained sections was done. From each paraffin block, four thin sections were taken on coated slides. Then those slides were stained with ER, PR, HER-2/Neu, and VEGF antibodies following the avidin-biotin-peroxidase staining method. The results of ER, and PR status in malignant cases were reviewed according to the Allred score.[11] The results of HER-2/ neu status were also reviewed according to the ASCO score.[12] The VEGF cytoplasmic staining of the tumor cells was scored by combining the percentage and the intensity of the stained tumor cells.[13,14] Statistical analysis was performed by applying the SPSS-PC package, version 23 (Statistical Package for Social Science).

Inclusion Criteria

- Histologically diagnosed cases of breast carcinoma.
- Paraffin blocks collected from the Department of Pathology, BIRDEM General Hospital, Dhaka from March 2018 to January 2020

Exclusion Criteria

- Cases taken prior chemotherapy or radiotherapy.
- Cases not collected from the specified hospital and period
- Cases not processed with the stated methods (ER, PR, HER-2/Neu, and VEGF staining)

RESULTS

Among the cases, demographic information on 44 individuals who were part of a study. The age range of the participants varies from 28 to 75 years, with the average age being 51.82 years old. The majority of the participants were female (95.6%) and postmenopausal (55.8%). A small proportion of the participants had a positive family history of breast cancer (8.9%). The involved breast was almost evenly split between the right and left (51.1% and 48.9%, respectively). The majority of the participants were married (93.3%).

Table 1: Demography of the study population

Demography of the study population	No. of Cases	Percent (%)
Age (Years)		
21-30	1	2.2
31-40	7	15.6
41-50	14	31.1
51-60	14	31.1
61-70	7	15.6
71-80	2	4.4
Mean ± SD (Min-Max)	51.82 ± 10.94 (28-75)	
Sex		
Male	2	4.4
Female	43	95.6
Menopausal history		
Premenopausal	19	44.2
Postmenopausal	24	55.8
Family history of breast cancer		
Positive	4	8.9
Negative	41	91.1
Laterality of the involved breast		
Right breast	23	51.1
Left breast	22	48.9
Marital status		

Married	42	93.3
Unmarried	3	6.7

Table 2: Correlation of immunohistochemical expression of VEGF with an expression of ER, PR, and overexpression of HER-2/neu

Expression of molecular markers	VEGF		p-value*
	Positive	Negative	
	ER		
Positive	17 (54.8%)	14 (45.2%)	0.293
Negative	10 (71.4%)	4 (28.6%)	
Total	27 (60.0%)	18 (40.0%)	
	PR		
Positive	13(54.2%)	11(45.8%)	0.393
Negative	14(66.7%)	7(33.3%)	
Total	27 (60.0%)	18 (40.0%)	
	HER-2/neu		
Positive	13 (59.1%)	9 (40.9%)	0.990
Weak positive	6 (60.0%)	4 (40.0%)	
Negative	8 (61.5%)	5(38.5%)	
Total	27 (60.0%)	18(40.0%)	

*Chi-square test was done to measure the level of significance.

This table shows the correlation between the immunohistochemical expression of VEGF and the expression of ER, PR, and overexpression of HER-2/neu. The p-value is the result of a chi-square test, which was used to measure the level of significance. A low p-value indicates a strong correlation. For example, a p-value of 0.293 for the correlation between VEGF and ER suggests a weak correlation between the two. The percentage of positive and negative expression is shown for each marker, with a total of 27 cases showing positive expression of VEGF and 18 cases showing negative expression.

Table 3: Correlation test of ER, PR, and HER-2/neu with VEGF

VEGF vs	r value	p-value
ER	-0.085	0.580
PR	-0.043	0.780
HER-2/neu	0.080	0.603

In this study the molecular subtype of breast carcinoma was defined according to the immunohistochemical expression of ER, PR, HER-2/neu that was proposed by Effi and his colleagues (2016), which is an adaptation of original classification. IS Among 45 cases, 10 cases were HER-2/neu weakly positive/equivocal. These HER-2/neu weakly positive or equivocal cases could not be further evaluated for gene amplification by Fluorescence in Situ Hybridization (FISH) due to unavailability of this test in our institute.

Table 4: Expression of VEGF in different molecular subtypes of breast carcinoma

IHC expression	VEGF		P-Value
	Positive (n=27)	Negative (n=18)	
Luminal A	3 (50.0%)	3 (50.0%)	0.670
Luminal B	15 (57.7%)	11 (42.3%)	0.702
Non luminal Her-2/neu positive	4 (66.7%)	2 (33.3%)	0.999
Triple-negative	5 (71.4%)	2 (28.6%)	0.684

Fisher's Exact test was done to measure the level of significance.

The data shows the number of patients positive for VEGF expression and the number of patients negative for VEGF expression in each subtype. The p-value for each subtype was calculated using Fisher's Exact Test, and the results suggest that there is no significant difference in the expression of VEGF between the subtypes (p-values ranging from 0.670 to 0.999).
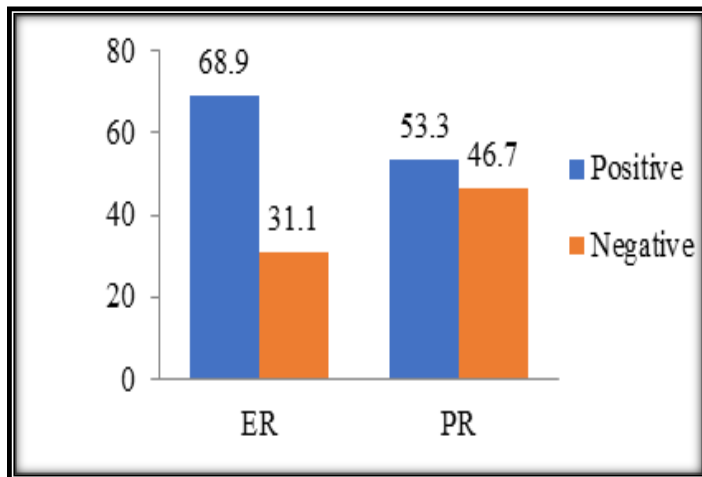
Figure 1: Bar diagram showing ER and PR expression in breast cancer.

This bar diagram shows the expression levels of ER (Estrogen Receptor) and PR (Progesterone Receptor) in breast cancer. The data shows that 68.9% of breast cancers are ER-positive and 31.1% cases are ER-negative. The expression level of PR shows that 53.3% of breast cancers are PR-positive and 46.7% of cases are PR-
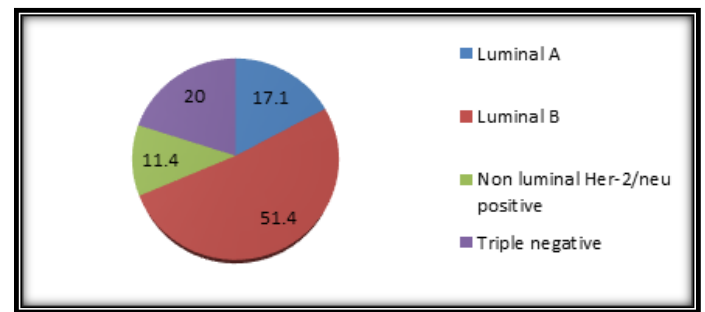
negative. 71.1% breast cancer cases are ER/PR positive.

Figure 2: Different molecular subtypes of breast carcinoma by immunohistochemistry
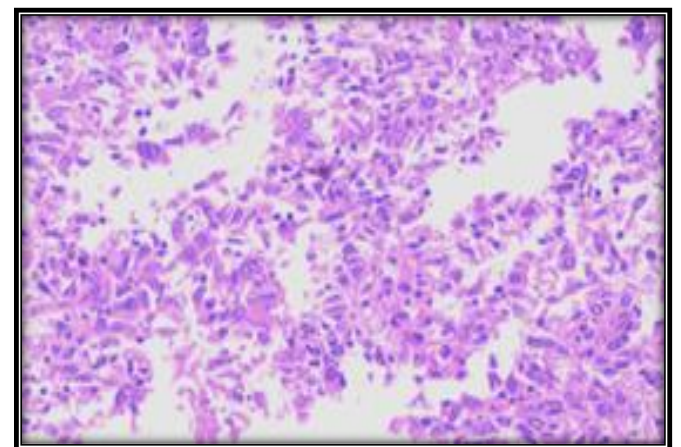

Figure 3: Photomicrograph of invasive ductal carcinoma (Grade-II) (H&E 40x).

The above figure lists the different molecular subtypes of breast carcinoma based on immunohistochemistry and their estimated

percentage of incidence in breast cancer cases. The four subtypes listed are Luminal A (17.1%), Luminal B (51.4%), Non-luminal Her-2/neu positive (11.4%), and Triple negative (20%). [Figure 2]
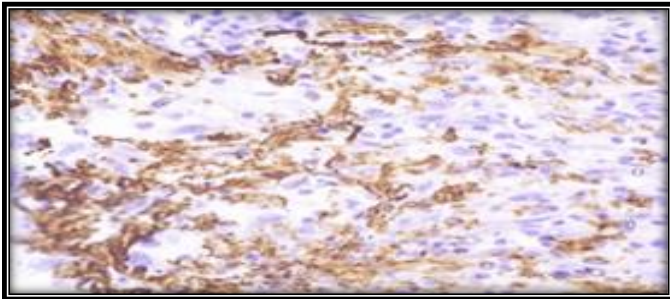

Figure 4: Photomicrograph of an invasive ductal carcinoma (Grade-II) showing positive VEGF expression (score: 6) (IHC 40x).
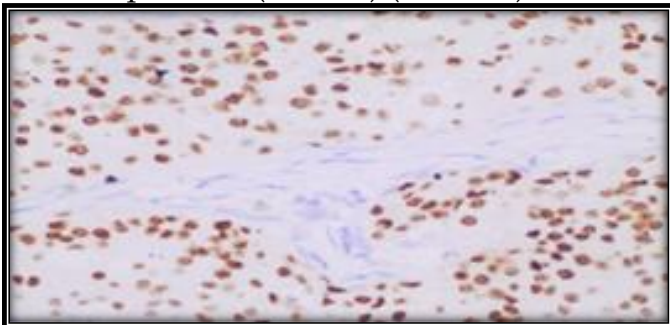

Figure 5: Photomicrograph of a ductal carcinoma (Grade-II) showing ER expression (score: 8) (IHC 40x).
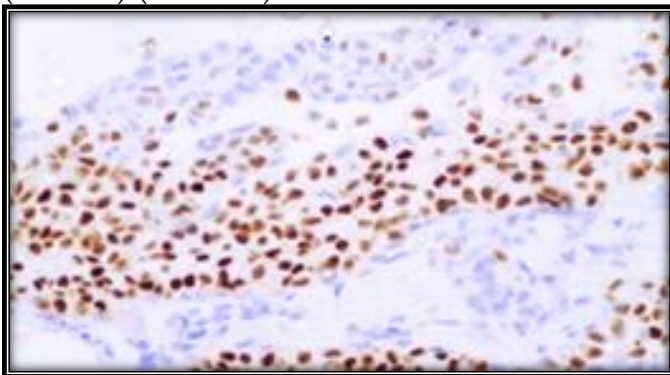

Figure 6: Photomicrograph of a ductal carcinoma (Grade-II) showing PR expression (score: 8) (IHC 40x).
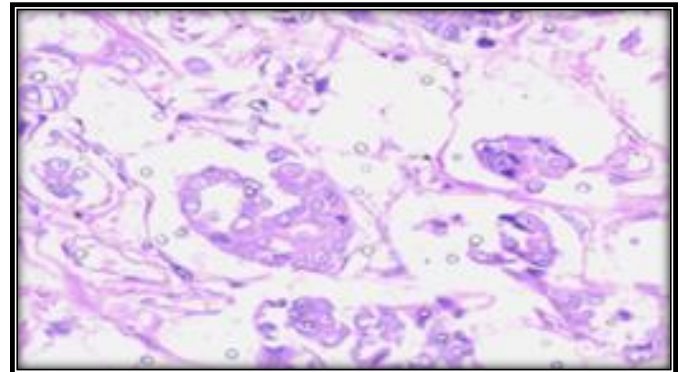

Figure 7: Photomicrograph of invasive ductal carcinoma (Grade-II) (H&E 40x).
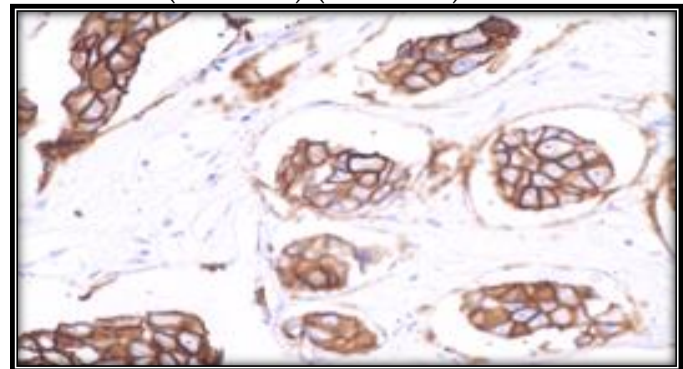

Figure 8: Photomicrograph of an invasive ductal carcinoma (Grade-II) showing Her-2/neu overexpression (score: 3+) (IHC 40x).

DISCUSSION

This cross-sectional study found out to observed the expression of VEGF in breast carcinoma and its association with the expression of ER, PR, and HER-2/neu in breast carcinoma in the Bangladeshi population. Most of the patients were of age between 41-60. About two-thirds of the patients presented with tumor size of 2.0-5.0 cm and 47.4% of patients presented with axillary lymph node metastasis at the time of diagnosis. In this study, the majority of tumors were determined to be grade II invasive carcinomas [Figure 3]. Approximately 60% of the cases were found to have a positive expression of VEGF. Several

studies were done in Egypt and Saudi Arabia showed similar findings.[15,16,17] Current studies showed that 68.9% of cases were ER-positive and 53.3% of cases were PR positive. ER-positive cases rate was similar to other studies conducted in our country; their ER-positive cases were 69.0%.[18] In this study, 71.1% (32) cases were ER/PR positive. In Germany, Konecny and his group carried out a study and determined that 77.6% of cases tested positive for ER/PR.[19] Our findings are quite similar to their findings. The association between the expression of ER, PR, and VEGF was evaluated in this study. In Pearson's correlation test, it was observed that the expression of VEGF increases with decreased expression of ER and PR. However, no significant correlation was found between them. It was consistent with other studies conducted in Japan and China.[20,21] HER-2/neu were found positive (score 3+) in 48.9% of cases, which was much higher than other studies conducted on Bangladeshi women and reported that HER-2/neu over-expressed tumor was 28.4%.[18] Mujtaba et al. (2013) reported that HER-2/neu is positive in 23.52% of cases in the Pakistani population.[22] Konecny and his workers (2004) also conducted a study in Germany and found that 18.7% of cases were HER-2/neu positive.[19] In India, a researcher reported that VEGF expression significantly correlated with the overexpression of HER-2/neu.[23] But, this study showed no such relationship. In this study, luminal A cases were 17.1%, luminal B cases were 51.4%, non-luminal HER-2/neu positive cases were 11.4% and triple-negative cases were 20.0%. In 2017, another researcher reported that luminal A, luminal B, and nonluminous Her-2 positive and triple negative cases were 25%, 22.73%, 29.55%, and 22.73% respectively among the Bangladeshi population.[24] The current study also evaluated the expression of VEGF among different molecular subtypes of breast cancer. 57.7% of cases of the luminal B subtype showed expression of VEGF and the majority (71.4%) of cases of the triple-negative subtype showed expression of VEGF. In conclusion, most of the breast carcinoma cases (60%) showed VEGF expression in this study. Despite the lack of a significant correlation between VEGF expression and the molecular markers ER, PR, and HER-2/neu. New therapeutic modalities can be tried against VEGF in breast cancer management. This study was not a population-based study, it was a center-based study. For that reason, we might not get the exact scenario. Further studies can be done on a larger scale.

CONCLUSIONS

Most of the breast carcinoma cases (71.1%) were ER/PR positive and the most common subtype is Luminal B (51.4%). Positive VEGF expression was found in 60% of breast cancer cases. So, VEGF may serve as a useful biomarker for immuno-targeting therapy in patients with breast cancer. The study population was small, that's why it could not draw significant findings. A further study on large scale is recommended to see the expression of VEGF in breast carcinoma and its relation with other prognostic markers.

Recommendation

Considering the results of this study, it is recommended to conduct further large-scale studies to confirm VEGF expression in breast carcinoma and its association with other prognostic markers such as ER, PR, and HER-2/neu in the Bangladeshi population. Additionally, the use of VEGF as a biomarker

for immuno- treatment that targets breast cancer patients should be evaluated in clinical trials. Further research in this field will help in the development of more effective therapeutic strategies for the management of breast cancer.

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