

## Original Article



# Immunohistochemical Expression of Vascular Endothelial Growth Factor in Different Histomorphological Types of Breast Carcinoma.

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### Abstract

**Background:** Breast carcinoma is a heterogenous disease that represent a major health problem in Bangladeshi population. Several molecular markers have been detected over the past several decades, that are important in clinical aspects of breast carcinoma. Most recently, Vascular Endothelial Growth Factor (VEGF) is emerging as a prognostic marker in patients with several types of carcinoma including breast carcinoma. Many anti-angiogenic therapies targeting VEGF has been proven effective in many carcinomas. **Objectives:** This study was aimed to determine the expression of VEGF in different histomorphological types of breast carcinoma in an attempt to clarify its potential clinical outcome. **Materials and Methods:** This study was conducted in the Department of Pathology, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) General Hospital, Dhaka from March 2018 to January 2020. In this study, 45 diagnosed cases of breast carcinoma were enrolled. Histopathological slides of all cases were re-evaluated. Thin sections were taken on coated slides from each paraffin block and immunohistochemical staining was done with VEGF antibody. **Results:** Among 45 cases, 73.3% cases were invasive ductal carcinoma and 13.3% cases were invasive lobular carcinoma. The immunohistochemical expression of VEGF was positive in 60% cases. In this study, 100% cases of in-situ ductal carcinoma, 83.3% cases of invasive lobular carcinoma and 57.6% cases of invasive ductal carcinoma showed overexpression of VEGF. However VEGF expression did not show significant association with histomorphological types. **Conclusion:** VEGF expression was found in 60% of breast carcinoma cases. It may serve as a potential therapeutic target for the management of breast carcinoma.

**Key words:** Angiogenesis, Breast carcinoma, Expression of Vascular Endothelial Growth Factor, Histomorphological Types, Immunohistochemistry.

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## Introduction

Globally, breast cancer is the most common malignant tumour. In 2020, there were 2.30 million women diagnosed with breast cancer and 685000 deaths were due to breast cancer. The incidence of breast cancer is 11.7% in both sexes. Its mortality declines gradually after 1980 due to advanced diagnostic and therapeutic methods in the developed countries, despite increasing or stable incidence rates.<sup>1</sup> Nevertheless, this cancer-related mortality remains very high in the developing countries because of late diagnosis and delay in treatment. Management and prognosis of breast carcinoma mainly depends on stage, grade, patient's age and expression of some molecular markers. Among different molecular markers ER, PR and HER-2/neu have been proven to have a great role in treatment and assessing prognosis of tumor. Patients with breast carcinoma are treated

with target therapy against ER, PR expression and HER-2/neu overexpression have been proven to prevent the relapse and progression of the tumor.<sup>2,3</sup>

Angiogenesis is an important step in the development of any carcinoma. Even if a solid tumor possesses all of the genetic aberration, that are required for malignant transformation, it cannot enlarge beyond 1 to 2 mm in diameter (due to lack of oxygen and nutrients) unless it has the capacity to induce angiogenesis by producing some proangiogenic factors.<sup>4</sup> Among the proangiogenic factors, VEGF is the most potent endothelial cell mitogen and acts as a central mediator of angiogenesis. VEGF promote growth of new blood vessels (irregularly shaped, tortuous, leaky and dilated capillary) from nearby capillaries and allows tumor to access the oxygen and nutrients which are necessary for its growth.<sup>5,6</sup> VEGF also plays an

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integral part in tumor growth through inducing the anti apoptotic factors (Bcl-2 and survivin), which prevent apoptosis of the newly formed vascular endothelial cells as well as the tumor cells.<sup>7</sup>

Several studies establish a significant correlation between VEGF expression and other known prognostic indicators. The expression of VEGF is found in breast cancer, colon carcinoma, renal cell carcinoma and glioblastoma. Therefore, VEGF expression could be an important marker of angiogenic activity for prognostic purposes as well as for targeting inhibition of angiogenesis as a novel therapeutic strategy against cancer. In 2004, anti-VEGF therapy (bevacizumab) has gained approval from the FDA for the treatment of metastatic colorectal carcinoma, renal cell carcinoma and glioblastoma. In those cases, it improves relapse-free survival period. Various therapeutic approaches aimed at inhibiting the function of VEGF in breast carcinoma are currently under investigation. Some are already in phase-III clinical trials and the effect of combining VEGF inhibitor with chemotherapeutic agents is also being studied.<sup>8,9</sup>

The aim of this study was to determine the level of expression of VEGF in breast carcinoma along with its association with different histomorphological types of breast carcinoma. Findings from this study may contribute to assess the role of anti-VEGF therapy for breast cancer patients.

### Materials and Method

The study material were 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. In this study, 45 diagnosed cases of breast carcinoma were enrolled. Re-evaluation of the routinely processed H&E stained sections were done.

From each paraffin block, thin sections (3-4 micrometer) were taken on coated slides. Then those slides were stained with VEGF antibody (Monoclonal Mouse Anti-Human Vascular Endothelial Growth Factor, Clone VG1, Code No. M7273, Dako Denmark) following the avidin-biotin-peroxidase staining method. The VEGF cytoplasmic staining (brown color) of the tumor cells were scored by combining the percentage and the intensity of the stained tumor cells.

<b>Score for proportion of positive stained cells :</b>	<b>Score for staining intensity:</b>
0= <10% of tumor cells are stained.	0= No Staining
1= 10-25% of tumor cells are stained.	1= Weak Staining/Light yellow
2= 25-50% of tumor cells are stained.	2= Moderate Staining / Brown
3= >50% of tumor cells are stained.	3= Strong Staining/Dark brown

The combined score was obtained by adding the individual scale of percentage of positive stained cells and the intensity of stained cell (range:0-6):

0-2= Negative staining (-); 3-4=Positive staining (+) & 5-6= Strong positive staining (++)<sup>10,11</sup>

Statistical analysis was performed applying the SPSS-PC

package, version 23 (Statistical Package for Social Science). P values less than 0.05 were considered significant. Analysis of the association between biomarker expression and clinicopathological parameters were performed by using Fisher’s exact test. The results were published in tables and pie charts.

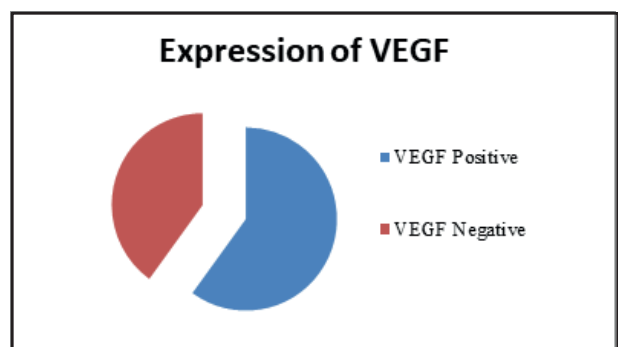
### Results

Among 45 cases, the mean age was 51.82±10.94 years with a female-male ratio of 21.5:1. Breast cancer was found more in postmenopausal women (55.8%). Only 4 (8.9%) cases had the family history of breast cancer. By light microscopy, diagnosed invasive carcinoma cases were 43 cases and only 2 (4.4%) cases were in situ carcinoma. Among invasive carcinomas, it was observed that more prevalent carcinoma was invasive ductal carcinoma (Figure 2) (73.3%) and second most common carcinoma was invasive lobular carcinoma (Figure 4) (13.3%) (Table I).

**Table I:** Distribution of the patients according to histomorphological type (n=45):

Histomorphological type	No. of Cases	Percent(%)
<b>Invasive carcinoma</b>		
o Invasive ductal carcinoma	33	73.3
o Invasive lobular carcinoma	6	13.3
o Mixed ductal and lobular carcinoma	4	8.9
<b>In situ carcinoma</b>		
o Ductal carcinoma in situ	2	4.4
<b>Total</b>	<b>45</b>	<b>100.0</b>

Immunohistochemical expression of VEGF were assessed in all cases. Among 45 cases, 27 (60.0%) cases were VEGF positive and 18 (40.0%) cases were VEGF negative (Figure 1). Using VEGF scoring system, among 27 VEGF Positive cases, it was found that 18 cases with score ++ (Figure 3) and 19 cases with score + (Figure 5).



**Figure 1:** Pie diagram showing VEGF expression in breast cancer.

In this study, 19 (57.6 %) cases of invasive ductal carcinoma were VEGF positive and 5 (83.3%) cases of invasive lobular carcinoma were VEGF positive. Both the cases (100%) of ductal carcinoma in situ (DCIS) were VEGF positive. However, no significant correlation was found in between the VEGF expression and different histomorphological types of the tumor (Table II).

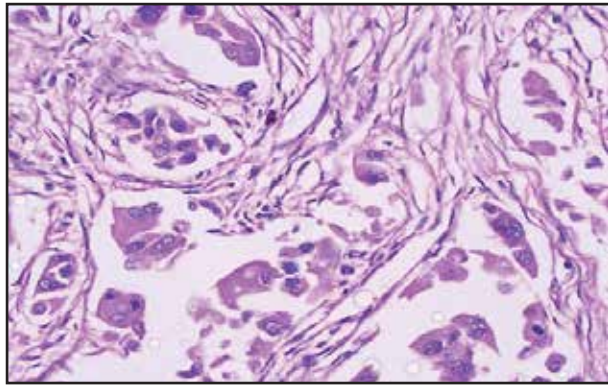
**Table II:** VEGF expression in different histomorphological types of breast cancer (n=45):

Histomorphological type	VEGF		p-value*
	Positive	Negative	
<b>Invasive carcinoma</b>			
○ Invasive ductal carcinoma	19 (57.6%)	14 (42.4%)	0.735
○ Invasive lobular carcinoma	5 (83.3%)	1 (16.7%)	0.377
○ Mixed ductal and lobular carcinoma	1 (25.0%)	3 (75.0%)	0.286
<b>In situ carcinoma</b>			
○ Ductal carcinoma in situ	2 (100.0%)	0 (0.0%)	0.509

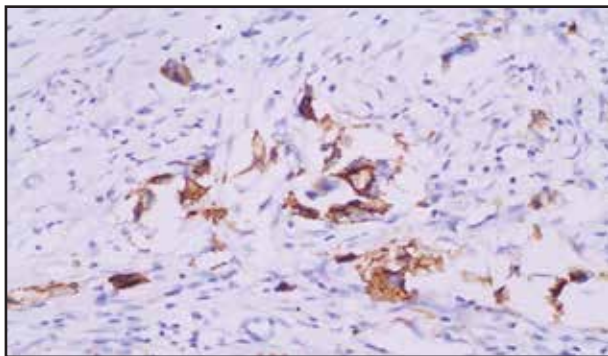
\*Fisher’s Exact test was done to measure the level of significance. Figure within parentheses indicated in percentage.

**Discussion**

Over the past several decades, Vascular Endothelial Growth Factor (VEGF) signaling has been identified as a central axis in tumor angiogenesis. Keeping this in mind, this cross sectional study was aimed to see the expression of VEGF in different histomorphological types of breast carcinoma in Bangladeshi population.

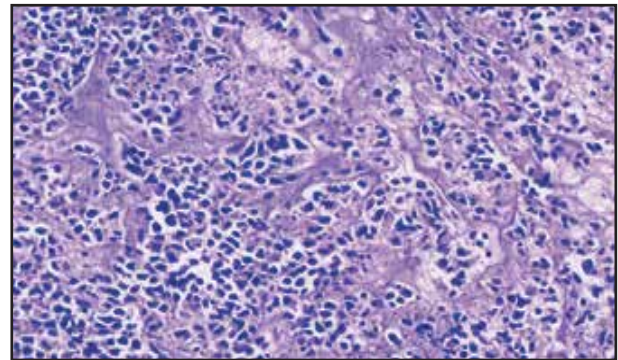


**Figure 2:** Photomicrograph of an invasive ductal carcinoma (Grade-II) (H&E 40x)

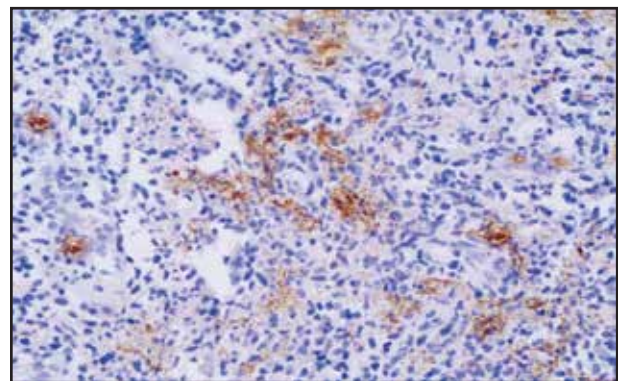


**Figure 3:** Photomicrograph of an invasive ductal carcinoma (Grade-II) showing positive VEGF expression (score: 6) (IHC 40x).

In this study, it was observed that 60% cases were positive and 40% cases were negative for VEGF. Different studies were conducted in different countries to see the expression of VEGF in breast carcinoma. Our finding was similar to other studies done in Egypt and Saudi Arabia, where the expression of VEGF in breast carcinoma was 60% and 61.5%.<sup>11,12</sup> Though expression of VEGF was found in 75% cases of breast carcinoma in Indian population, which is slightly higher than our study.<sup>13</sup>



**Figure 4:** Photomicrograph of an invasive lobular carcinoma (Grade-I) (H&E, 40x).



**Figure 5:** Photomicrograph of an invasive lobular carcinoma (Grade-I) showing positive VEGF expression (score: 4) (IHC, 40x).

The most common histomorphological type of breast carcinoma was invasive ductal carcinoma (73.3%) followed by invasive lobular carcinoma (13.3%). In 2016, Ragab and his colleagues conducted a study on Egyptian population and reported that, 86% cases were invasive ductal carcinoma and 8.7% cases were invasive lobular carcinoma. Findings were quite similar to this study.<sup>11</sup> About 57.6% cases of invasive ductal carcinoma and 83.3% cases of invasive lobular carcinoma showed positive expression of VEGF. 100.0% Cases of DCIS showed VEGF expression. This can be explained by that, VEGF play an important role in cancer progression or promotion (from in situ to invasive form) and in advanced cases the VEGF expression was reduced due to the expression of other angiogenic factors (like platelet-derived endothelial growth factor and transforming growth factor). However, no significant correlation was found in between the expression of VEGF and histomorphological types of breast carcinoma. This finding was

consistent with other studies conducted in Egypt and Australia.<sup>11,14</sup> However, another study was conducted in Iraq where they found a significant association of the VEGF expression with invasive ductal carcinoma.<sup>15</sup>

Finally, most of the breast carcinoma cases (60%) showed VEGF expression. Though, no significant correlation was found in between the VEGF expression and histomorphological types of breast carcinoma. Based upon the findings of current study, it can be understood that, VEGF plays an important role in the pathogenesis of breast cancer. So, it may serve as a potential therapeutic target for the management of breast carcinoma.

1. This was not a population based study, it was a single centre based study. The study population was small, that's why it could not draw significant findings.
2. Lack of advanced techniques.

Further study on large scale is recommended to see the expression of VEGF in breast carcinoma and its relation with other prognostic markers along with the therapeutical trials against VEGF.

## Conclusion

Positive VEGF expression was found in 60% of breast cancer cases. So, target therapy against VEGF may consider for better outcome specially in those patients, whose response to conventional therapy is poor.

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## References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*. 2021 May; 71(3): 209-249.
2. Jung SU, Sohn G, Kim J, Chung IY, Lee JW, Kim HJ, Ko BS, Son BH, Ahn SH, Yang SW, Lee SB. Survival outcome of adjuvant endocrine therapy alone for patients with lymph node-positive, hormone-responsive, HER2-negative breast cancer. *Asian journal of surgery*. 2019 Oct 1; 42(10): 914-921.
3. Niikura N, Shimomura A, Fukatsu Y, Sawaki M, Ogiya R, Yasojima H, Fujisawa T, Yamamoto M, Tsuneizumi M, Kitani A, Watanabe J. Durable complete response in HER2-positive breast cancer: a multicenter retrospective analysis. *Breast cancer research and treatment*. 2018 Jan; 167(1): 81-87.
4. Carmeliet P. VEGF as a key mediator of angiogenesis in cancer. *Oncology*. 2005; 69(Suppl. 3): 4-10.
5. Gasparini G. Prognostic value of vascular endothelial growth factor in breast cancer. *The oncologist*. 2000 Apr; 5: 37-44.
6. Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. *Nature reviews cancer*. 2003 Jun; 3(6): 401-410.
7. Barr MP, Bouchier-Hayes DJ, Harmeij JJ. Vascular endothelial growth factor is an autocrine survival factor for breast tumour cells under hypoxia. *International journal of oncology*. 2008 Jan 1; 32(1): 41-48.
8. Kong DH, Kim MR, Jang JH, Na HJ, Lee S. A review of anti-angiogenic targets for monoclonal antibody cancer therapy. *International journal of molecular sciences*. 2017 Aug; 18(8): 1786.
9. Paradis V, Lagha NB, Zeimoura L, Blanchet P, Eschwege P, Ba N, Benoît G, Jardin A, Bedossa P. Expression of vascular endothelial growth factor in renal cell carcinomas. *VirchowsArchiv*. 2000 Apr; 436(4): 351-356.
10. Shehata SM, Mooi WJ, Okazaki T, El-Banna I, Sharma HS, Tibboel D. Enhanced expression of vascular endothelial growth factor in lungs of newborn infants with congenital diaphragmatic hernia and pulmonary hypertension. *Thorax*. 1999 May 1; 54(5): 427-431.
11. Ragab HM, Shaaban HM, El Maksoud NA, Radwan SM, Elaziz WA, Hafez NH. Expression of vascular endothelial growth factor protein in both serum samples and excised tumor tissues of breast carcinoma patients. *Int. J. Cancer Res*. 2016; 12: 152-161.
12. Al-Harris ES, Al-Janabi AA, Al-Toriahi KM, Yasseen AA. Over expression of vascular endothelial growth factor in correlation to Ki-67, grade, and stage of breast cancer. *Saudi Med J*. 2008 Aug 1; 29(8): 1099-1104.
13. Bhat S, Tania RP, Hussain S, Sahaf B, Ansari I. Expression of VEGF in breast lesions: An immunohistochemical study. *International Journal of Advances in Medicine*. 2019; 6: 12-17.
14. Obermair A, Kucera E, Mayerhofer K, Speiser P, Seifert M, Czerwenka K, Kaider A, Leodolter S, Kainz C, Zeillinger R. Vascular endothelial growth factor (VEGF) in human breast cancer: correlation with disease-free survival. *International journal of cancer*. 1997 Aug 22; 74(4): 455-458.
15. Almumen M. Immunohistochemical Expression of VEGF in Relation to Other Pathological Parameters of Breast Carcinoma. *Journal of Cancer Therapy*. 2015; 6(09): 811.