ORIGINAL RESEARCH

A Study to Correlate Vascular Endothelial Growth Factor(Vegf) Expression in Cases of Invasive Breast Carcinoma with Estrogen Receptor(Er), Progesterone Receptor(Pr), Her-2/Neu And Ki- 67 Index Expression by Immunohistochemical(Ihc) Markers

[1]Dr. Abhirup Khastagir, [2]Dr. Nirmal Kumar Bhattacharyya

1Senior Resident, Department of pathology, Barasat Government Medical College and Hospital
2Associate professor, Department of pathology, Jalpaiguri Government Medical College

Corresponding author
Dr. Abhirup Khastagir
Senior Resident, Department of pathology, Barasat Government Medical College and Hospital

ABSTRACT
Introduction: Breast cancer is the most frequent and lethal tumour in women worldwide, with roughly 1.7 million women diagnosed each year and one in every three dying from the disease. Although the incidence of breast cancer is 4-7 times greater in the United States and Europe than elsewhere, global incidence and death are increasing at an alarming rate, and it is anticipated that by 2020, 70% of cases will be in low-income nations. The study's goal is to link Vascular Endothelial Growth Factor (VEGF) expression with ER, PR, Her-2/Neu, and Ki- 67 index expression in cases of invasive breast cancer using immunohistochemistry (IHC).
Materials and Methods: FFPE section of 30 cases of HPE confirmed cases of breast malignancy were taken up and subjected to IHC using predetermined markers.
Result: The intensity of VEGF immunostaining in malignant breast lesions was evaluated
and scoring was done and evaluated as positive($\geq$3) or negative($<$3). Statistical analysis was performed with Chi-Square test. And significant differences were noted between different variables.
Conclusion: Our study showed a statistically significant association between VEGF expression and ER and Her2/neu expression. Also VEGF expression was negatively correlated to Her2/neu expression.
Keywords: Breast cancer, Survival, Vascular endothelial growth factor and Angiogenesis.

Introduction

In India, breast cancer accounts for about 25- 31% of all the cancers in women. The average age of developing breast cancer has shifted recently to 30-50 years. [2]

All the breast cancers can be separated into three major groups defined by the expression of two proteins, ER and Her 2/ neu. [1]

• Hormone therapy medicines that lower oestrogen levels or inhibit oestrogen receptors can be used to treat hormone receptor positive breast cancer. Cancers that express hormone receptors grow more slowly. Women with hormone receptor positive malignancies had a better prognosis in the short term, however these tumours can recur many years later.[3]

• Hormone therapy medications are ineffective in the treatment of hormone receptor negative breast tumours. These malignancies grow more quickly than hormone receptor positive tumours. If they reappear following therapy, it is usually in the first few years and is more likely in women who have not yet reached menopause.[3]

• Triple negative breast cancer is more likely among women under the age of 40, who are black, or who have a BRCA1 gene mutation. Because hormone treatment is ineffective in treating these malignancies, triple negative breast cancers develop and spread quicker than most other kinds of breast cancer.[3]

• Triple positive cancers can be treated with hormone and drugs.

• The Oestrogen Receptor (ER) is a transcription factor that is activated by ligand. Oestrogen and other steroid hormones bind to and activate ER, signalling its translocation to the nucleus. Once in the nucleus, activated ER can bind DNA and induce transcription of genes involved in a variety of cellular functions such as proliferation, apoptosis evasion, invasion, and angiogenesis. [4]

• The Progesterone Receptor (PR) is a ligand-dependent transcription factor that belongs to the nuclear hormone receptor family. Steroid hormones bind to and activate PR, causing transcription of

genes involved in a variety of cellular activities that promote growth and invasion.

PR is a biomarker that is regularly employed at the time of diagnosis to characterise breast cancer. PR is found in more than 50% of ER positive breast tumours but is rarely found in ER negative breast tumours.[5]

- Her 2/ neu is a member of the Epidermal Growth Factor (EGF) family, which also includes Her1 (erbB1), Her2 (erbB2), Her3 (erbB3), and Her4 (erbB4) receptors. The Her2 gene is found on chromosome 17's long arm and encodes an 185 Kilo Dalton transmembrane protein.[6]
- Antigen Ki-67 Ki-67 is a protein that is encoded by the MKI 67 gene in humans. Ki- 67 is active throughout the cell cycle's G1, S, G2, and M phases, making it an excellent marker of cell proliferation and an acknowledged hallmark of oncogenesis.[7]
- VEGF- Originally known as Vascular Permeability Factor (VPF), is a signal protein produced by the cells that stimulate the formation of blood vessels.[8]

Solid cancers cannot grow beyond a limited size without adequate blood supply; cancers that can express VEGF are able to grow and metastasize.

Materials and Methods

The prospective observational study was conducted at a tertiary care hospital of Kolkata. All patients taken up for study were female with breast lump irrespective of their age and other physical conditions during the period of January 2021 to June 2022. 30 cases were selected, all H&E confirmed cases of malignant breast lesions.

The corresponding FFPE blocks were subjected to IHC using ER, PR, Her2/neu, VEGF antibody.

ER, PR scoring was done using standard Allred scoring system.[8]

Her2/neu scoring was done using standard criteria.[8]

Ki- 67 SCORING SYSTEM:- In our study, the cut-off point for Ki-67 status was more than 14% of positively stained cells, which was in correlation with that of Fasching *et al*. Cheang *et al*(2009).[9], Yerushalmi *et al*(2010)[10] and Cheang *et al*(2009).

VEGF SCORING SYSTEM:- Accoarding to the study by Han H, Wang B, Zhao J *et al* (2017)[11], VEGF is detected in the cytoplasm. Expression of VEGF is evaluated and scored as follows :

1. % of positive cells are quantified by using the below scoring system:

% of positive staining cells	Score
< 10%	1
>10-50%	2
>50%	3

2. Staining intensity scoring is as follows:

colour intensity	Points accredited
no colour	0
Uniform yellow background	1
Brown	2
Tan	3

Finally, the combination of the above 2 values are used for assessing the positivity of the stain-

≥3	positive expression
<3	negative expression

Statistical analyses of all results were done by using Chi square test.

Ethical clearance was obtained from the Ethical Committee Meeting conducted at the respective tertiary care hospital of Kolkata.

Result

In VEGF positive cases, 7 (29.2%) patients were ≤40 years of age, 7 (29.2%) patients were 41-50 years of age, 6 (25.0%) patients were 51-60 years of age and 4 (16.7%) patients were 61-70 years of age. Association of Age in years with VEGF expression was not statistically significant (p=0.9778).

In VEGF Group, 16 (66.7%) patients had ER positivity. Association of ER with VEGF was statistically significant (p=0.0270). In VEGF Group,

13 (54.2%) patients had PR positivity. Association of PR with VEGF was not statistically significant (p=0.3613). In VEGF Group, 5 (9.5%) patients had Her2/neu positivity. Association of Her2/neu with VEGF was statistically significant (p=0.0062).

In VEGF Group, 15 (62.5%) patients had High Ki-67 expression and 9 (37.5%) patients had Low Ki-67 expression. Association of Ki-67with VEGF was not statistically significant (p=0.1972).

1369

Table 1: Association between ER, PR and Her2/neu :VEGF

		VEGF			Chi-square value	p-value
ER		Negative	Positive	TOTAL		
	Negative	5	8	13	4.8869	0.0270
		38.5	61.5	100.0		
		83.3	33.3	43.3		
	Positive	1	16	17		
		5.9	94.1	100.0		
		16.7	66.7	56.7		
	TOTAL	6	24	30		
		20.0	80.0	100.0		
		100.0	100.0	100.0		
PR	Negative	4	11	15	0.8333	0.3613
		26.7	73.3	100.0		
		66.7	45.8	50.0		
	Positive	2	13	15		
		13.3	86.7	100.0		
		33.3	54.2	50.0		
	TOTAL	6	24	30		
		20.0	80.0	100.0		
		100.0	100.0	100.0		
Her2/neu	Negative	4	19	23	7.4623	0.0062
		17.4	82.6	100.0		
		44.4	90.5	76.7		
	Positive	2	5	7		
		71.4	28.6	100.0		
		55.6	9.5	23.3		
	TOTAL	6	24	30		
		30.0	70.0	100.0		
		100.0	100.0	100.0		

Table 2: Association between Ki-67: VEGF

	VEGF		
Ki-67	Negative	Positive	TOTAL
High	2	15	17
	11.8	88.2	100.0
	33.3	62.5	56.7
Low	4	9	13
	30.8	69.2	100.0
	66.7	37.5	43.3
TOTAL	6	24	30
	20.0	80.0	100.0
	100.0	100.0	100.0

Discussion

Cimpean A.M, Raica M *et al* **(2008)** [12] in their research discovered a link between invasive ductal carcinoma of the breast and VEGF expression. They included 35 invasive breast cancer cases that were immunostained for VEGF with a monoclonal antibody, anti-VEGF.

The assessment of VEGF expression was done using a scoring system which included an intensity parameter correlated with the percentage of positive tumor cells. They found a positive correlation between invasive ductal carcinoma of breast and VEGF expression.

In our study 30 patients were taken based on crude census method. We used the scoring system for assessment of VEGF expression as given by the study by **Han H, Wang B, Zhao J** *et al* **(2017)** [11].

Adam J, Carder P.J. *et al* **(2000)** [15] in their research found a high link between VEGF expression and oestrogen receptor status.

Our study also shows similar findings in that, In VEGF expressing group, 16 (66.7%) patients had ER positivity. Thus association of ER with VEGF was statistically significant (p=0.027).

Bhat S, Tania R.P. *et al*(2019) [14], **Cimpean A.M ,Raica M** *et al* **(2008)**[12] in their study showed There was no significant relationship between VEGF expression and the age and menopausal status of the women in their study.

Our study also shows similar results- In VEGF positive cases, 7 (29.2%) patients were ≤40 years of age, 7 (29.2%) patients were 41-50 years of age, 6 (25.0%) patients were 51-60 years of age and 4 (16.7%) patients were 61-70 years of age.

Thus, association of Age in years with VEGF was not statistically significant (p=0.97).

Mais Almumen *et al*(2015)[16] showed In our investigation, there was no significant variation in VEGF expression between age groups.

VEGF expression, on the other hand, exhibited a statistically significant positive connection with Her-2/neu expression. This contradicts our findings, which demonstrated a statistically significant but inverse link between VEGF and Her-2/neu expression.

They also found an inverse correlation of VEGF expression with ER, PR expression. This again contradicts with our study which shows a positive statistically significant correlation of VEGF with ER expression.

The study done by Liu Ying, Tamimi. M. Rulla *et al*(2011)[13], showed that VEGF expression was found in 72.5% of invasive breast tumours. The study discovered a link between VEGF and Her-2/neu. VEGF, on the other hand, has a substantial negative connection with ER and PR expression.

VEGF positive was found in 24 (80.0%) of the patients in our investigation. VEGF, on the other hand, has a high positive association with ER expression but no statistically significant correlation with PR expression.

Contradicting to our study, Fuckar D *et al* **(2006)** [18]; in their study showed a significantly negative correlation between ER and VEGF expression.

Paola D, F Granato *et al* **(2002)** [19], in their study showed in various incidences of aggressive breast cancer, VEGF expression ranged from 0% to 95%. Younger (60 years) individuals had considerably higher VEGF expression than older patients. Furthermore, VEGF expression did not correlate with ER or PR receptor status.

Similar findings were also observed in our study with respect to age and PR expression.

Study by Li B-J, Zhu Z-H *et al* **(2004)**[20] showed that in cases of invasive breast cancer, Ki-67 expression demonstrated no significant connection with VEGF expression. Similarly, the association of Ki-67 with VEGF was not statistically significant (p=0.197) in our study.

Kushlinskii E. N. *et al* **(2004)** [21] in their study On invasive breast cancer patients, researchers discovered that high VEGF expression in tumour cells was associated with a high Ki-67 level. This runs counter to the conclusions of our investigation.

Linderholm K. B., Hellborg H *et al*(2011)[22]did a study involving individuals with invasive steroid receptor positive breast cancer. They had strong VEGFR-2 expression, which corresponded well with increased VEGF expression, as well as negative ER and PR expression.

These findings correlated well with the findings of our study.

Rydén L, Jirström K *et al*(2005) [23]in their study showed that Tamoxifen treatment showed no effect on patients with high VEGF expressing tumours. These high VEGF-expressing instances had a statistically significant connection with positive ER and PR expression in tumour cells.

They found no significant correlation between VEGF expression and Her2/ neu status of invasive breast cancer cases. Their study included only cases of premenopausal women with invasive breast cancers. They concluded that Tamoxifen in combination with VEGF inhibitor might be a novel treatment approach for VEGF expressing invasive breast cancers, and such a combined treatment might restore the Tamoxifen response in resistant cases of invasive breast cancer.

Al-Harris ES, Al-Janabi AAA, Al-Toriahi KM *et al* **(2008)** [24] in their study showed that VEGF expression was positively correlated with Ki-67 expression and recurrence of breast cancer.

The study recommended that the blocking of VEGF may be a target for blocking angiogenesis and hence improving the efficacy of anti-cancer therapy.

Conclusion

➢ Breast carcinoma is the most frequent and lethal tumour in women worldwide, with 1.7 million women diagnosed each year and one in every three dying from the disease. Breast cancer develops a decade sooner in India, with a higher proportion of young breast cancer patients than in Western countries.

➢ In India, breast cancer accounts for about 25-31% of all the cancers in women.

➢ Almost 50% of all breast cancers in India occur in premenopausal women. Patients younger than 60 years of age account for more than 80% of breast cancer cases in India.

- In India 30-45% of patients present with locally advanced breast cancer and metastatic disease.
- Compared with western populations, India also has a higher proportion of Her 2/neu positive and triple negative breast cancers.
- We studied 30 cases of Invasive Breast Carcinoma.
- We studied the expression of immunohistochemical markers (ER, PR, Her 2/ neu, ki67) in all 30 cases and tried to correlate them with the expression of a new immunohistochemical marker VEGF and thus analysing the statistical significance of the correlation.
- Among 30 cases, age of the patients varied from 36 - 80 years with mean age being 50 years and 60% patients presenting below the age of 50 years.
- In our study all the patients were female (n=30, 100%).
- In VEGF positive group, 16 (66.7%) patients had ER expression.
- Thus association of ER with VEGF was positively correlated and statistically significant (p=0.027).
- In VEGF positive Group, 5 (9.5%) patients had Her2/neu expression.
- Thus association of Her2/neu with VEGF was negatively correlated but statistically significant (p=0.006).
- On the other hand, In VEGF positive group, 13 (54.2%) patients had PR expression.
- Thus association of VEGF with PR was not statistically significant (p=0.3613).
- Also, in VEGF Group, 15 (62.5%) patients had High Ki-67 and 9 (37.5%) patients had Low Ki-67.
- Thus association of VEGF with Ki-67 was not statistically significant (p=0.1972).

References

1. Lester Sc. The Breast. In: Abbas AK, Aster JC, Kumar V (Eds). Robbins and Cotran Pathologic Basis of Disease. 10th ed. Philadelphia: Elsevier; 2020. p l067 -1064.
2. https://pa.nhp.gov.in/disease/reproductive-system/female-gynaecological-diseases-/breast-cancer-carcinoma
3. https://www.cancer.org/cancer/breast-cancer/understanding-a-breast-cancer diagnosis/breast-cancer-hormone-receptor-status.html
4. https://oncologypro.esmo.org/education-library/factsheets-on-biomarkers/er-in-breast-cancer
5. https://oncologypro.esmo.org/education-library/factsheets-on-biomarkers/pr-in-breast-cancer
6. Mitri Z, Constantine T, O'Regan R. The HER2 receptor in breast cancer: Pathophysiology, clinical use, and new advances in therapy. Chemother Res Pract . 2012 ;2012:743193.
7. Davey MG, Hynes SO, Kerin MJ, Miller N, Lowery AJ. Ki-67 as a prognostic biomarker in invasive breast cancer. Cancers (Basel). 2021;13(17):4455.
8. Muin S.A.Tuffaha, Hans Guski, Glen Kristiansen. Immunohistochemistry in Tumor Diagnostics. 1st ed. 2018. Cham, Switzerland: Springer International Publishing; 2017.
9. Cheang MCU, Chia SK, Voduc D, Gao D, Leung S, Snider J, et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst. 2009 ;101(10):736–50.
10. Yerushalmi R, Woods R, Ravdin PM, Hayes MM, Gelmon KA. Ki67 in breast cancer: prognostic and predictive potential. Lancet Oncol. 2010;11(2):174–83.
11. H H, B W, J Z, G X, X W, F C. Analysis of SHIP2 expression and its correlation with VEGF and HER-2 expression in breast cancer. J Clin Exp Pathol . 2017 ;07(04):1–6.
12. Cîmpean AM, Raica M, Suciu C, Tătucu D, Sârb S, Mureşan AM. Vascular endothelial growth factor A (VEGF A) as individual prognostic factor in invasive breast carcinoma. Rom J Morphol Embryol. 2008;49(3):303–8.
13. Liu Y, Tamimi RM, Collins LC, Schnitt SJ, Gilmore HL, Connolly JL, et al. The association between vascular endothelial growth factor expression in invasive breast cancer and survival varies with intrinsic subtypes and use of adjuvant systemic therapy: results from the Nurses' Health Study. Breast Cancer Res Treat. 2011;129(1):175–84.
14. Bhat S, P. TR, Hussain S, Sahaf B, Ansari I. Expression of VEGF in breast lesions: an immunohistochemical study. Int J Adv Med . 2019 ;6(1):12.
15. Adams J, Carder PJ, Downey S, Forbes MA, MacLennan K, Allgar V, et al. Vascular endothelial growth factor (VEGF) in breast cancer: Comparison of plasma, serum, and tissue VEGF and microvessel density and effects of Tamoxifen1. Cancer Res. 2000; 60(11):2898–905.
16. Reeves KW, Ness RB, Stone RA, Weissfeld JL, Vogel VG, Powers RW, et al. Vascular endothelial growth factor and breast cancer risk. Cancer Causes Control . 2009 ;20(3):375–86.
17. Yavuz S, Paydas S, Disel U, Zorludemir S, Erdogan S. VEGF-C expression in breast cancer: clinical importance. Adv Ther. 2005;22(4):368–80.
18. Fuckar D, Dekanić A, Stifter S, Mustać E, Krstulja M, Dobrila F, et al. VEGF expression is associated with negative estrogen receptor status in patients with breast cancer. Int J Surg Pathol. 2006;14(1):49–55.
19. De Paola F, Granato AM, Scarpi E, Monti F, Medri L, Bianchi S, et al. Vascular endothelial growth factor and prognosis in patients with node- negative breast cancer. Int J Cancer. 2002;98(2):228–33.
20. Li B-J, Zhu Z-H, Wang J-Y, Hou J-H, Zhao J-M, Zhang P-Y, et al. Expression correlation of Ki67 to P53,VEGF,and C-erbB-2 genes in breast cancer and their clinical significances. Ai Zheng. 2004;23(10):1176–9.
21. Kushlinskii NE, Orinovskii MB, Gurevich LE, Kazantseva IA, Talaeva SZ, Shirokii VP, et al. Expression of biomolecular markers (Ki-67, PCNA, Bcl-2, BAX, BclX, VEGF) in breast tumors. Bull Exp Biol Med. 2004;137(2):182–5.

22. Linderholm BK, Hellborg H, Johansson U, Skoog L, Lehtiö J. Vascular endothelial growth factor receptor 2 and downstream p38 mitogen-activated protein kinase are possible candidate markers of intrinsic resistance to adjuvant endocrine treatment in steroid receptor positive breast cancer. Breast Cancer Res Treat . 2011;125(2):457–65.

23. Rydén L, Jirström K, Bendahl P-O, Fernö M, Nordenskjöld B, Stål O, *et al*. Tumor-specific expression of vascular endothelial growth factor receptor 2 but not vascular endothelial growth factor or human epidermal growth factor receptor 2 is associated with impaired response to adjuvant tamoxifen in premenopausal breast cancer. J Clin Oncol . 2005;23(21):4695–704.

24. Al-Harris ES, Al-Janabi AAA, 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;29(8):1099–104.