Original Article

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Expression of VEGF-C in Breast Carcinoma and its Association with HER2/neu and Oestrogen/ Progesterone Receptors: A Cross-sectional Study

Pathology Section

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ABSTRACT

Introduction: Breast carcinoma is the most common malignant tumour and is the leading cause of carcinoma death in women. Assessing Vascular Endothelial Growth Factor-C (VEGF-C) is important because the expression of VEGF-C is one of the factors determining the aggressiveness of breast carcinoma.

Aim: To evaluate the expression of VEGF-C in breast carcinoma and its association with HER2/neu and Oestrogen Receptors (ER)/Progesterone Receptors (PR).

Materials and Methods: A cross-sectional observational study was carried out in Department of Pathology of a tertiary care center of Burdwan Medical College and Hospital, West Bengal from 1st January 2018 to 30th June 2019. Patients attending Outpatient Department (OPD) and were diagnosed clinically as having breast cancer, operated upon, and subsequently sent for Histopathological Examination (HPE) in Pathology. The samples which were positive for breast carcinoma by histopathology were further studied with Immunohistochemical (IHC) markers and the positivity of IHC expression was reported using standard procedure and scoring pattern. The assosciation of VEGF-C expression with respect to histological type, grade, and stage of cancer as well as the HER2/neu and ER/PR status was studied. Using the Statistical Package for Social Sciences (SPSS) version 18.0, Chi-square was applied for the significance of p-value and the significance level was considered at a p-value <0.05.

Results: This study consisted of 44 cases of malignant breast lesions and of which 27 cases were associated with axillary lymph nodes. After IHC staining, 25 (56.82%) were VEGF-C positive and the rest 19 (43.18%) were VEGF-C negative. There was a significant association between VEGF-C and histologic grades of breast carcinoma. VEGF-C expression was significantly associated with lymph node metastasis and with HER2/neu. VEGF-C seems to be significantly associated with ER status of breast carcinoma and was not associated with the PR status of breast carcinoma.

Conclusion: Assessment of VEGF-C is recommended because expression of VEGF-C is one of the factors determining the aggressiveness of breast carcinoma. If VEGF-C is positive in node-negative breast carcinoma, then it may help in targeted therapy of breast carcinoma before lymphatic metastasis develops.

Keywords: Lymphovascular Invasion, Prognostic markers, Node

INTRODUCTION

The incidence of breast cancer is increasing throughout the world and it has become a major health problem. In India, breast carcinoma is the 2nd most common malignancy in females after cervical cancer. Breast cancer has ranked number one cancer among Indian females with age adjusted rate as high as 25.8 per 100,000 women and a mortality of 12.7 per 100,000 women [1].

Among the prognostic factors, microscopic grading is one of the most important factors. Histological grading of breast carcinoma was first attempted in 1925 by Greenough RB by using variations of morphological appearances of tumours [2]. It was shown quite early that survival is related to histological grade and this was confirmed by Bloom [3,4]. Lymph node metastasis is one of the most important factors predicting survival in patients without distant metastasis. Lymphovascular Invasion (LVI) is strongly associated with lymph node metastasis and is indicative of a poor prognosis. VEGF stimulates the growth of new blood vessels. VEGF-C plays a major role in lymphangiogenesis and metastasis to the lymph node. It can be detected by IHC in tissue sections [5].

Among the other factors, IHC markers like OR and PR and HER2/neu significantly modify the prognosis. In breast cancer, tumour grading and axillary lymph node metastasis are very important from the prognostic point of view [5]. There are several of these markers like growth factor receptors, steroid receptors, Ki-67, p21, cyclins, urokinase, plasminogen activators, p53, pro and anti-apoptotic factors, BRCA1 and BRCA2 which are prognostic groups of markers. Examples of predictive biomarkers are Hormonal receptors, PR and HER2/neu. ER/PR positive cases respond to endocrine treatment like tamoxifen and HER2/neu positive cases respond to Herceptin/Trastuzumab. But still, there are numerous breast cancer cases, where tamoxifen is ineffective even after ER is positive [6]. Very few studies assessing the correlation of VEGF-C and HER2/neu and ER/PR status have been done [5-9]. Biomarkers are necessary for the prognosis and prediction of chemotherapy for breast carcinoma. These biomarkers are of 2 types- prognostic and predictive. Prognostic biomarkers provide information regarding outcome irrespective of treatment and predictive biomarkers provide information regarding response to treatment [9]. So, the search for new prognostic and predictive biomarkers became necessary. VEGF and their receptors are important among those biomarkers.

Assessing VEGF-C is important because the expression of VEGF-C is one of the factors determining the aggressiveness of breast carcinoma. If VEGF-C is positive in node-negative breast carcinoma, then it may help in targeted therapy of breast carcinoma before lymphatic metastasis develops. The aim of this study was to evaluate the expression of VEGF-C in breast carcinoma and its association with HER2/neu and ER/PR. The specific objective of this study was to the assosciation of VEGF-C expression in breast carcinoma with the grading of carcinoma breast and its association with metastasis to the axillary lymph nodes.

MATERIALS AND METHODS

This cross-sectional descriptive study was conducted in the Department of Pathology in collaboration with the Department

of Surgery, Burdwan Medical College and Hospital, West Bengal from 1st January 2018 to 30th June 2019. After obtaining Ethical Clearance from Institutional Ethical Committee (IEC no. BMC/PG/1167) and informed consent from the subjects, study was initiated.

Inclusion criteria: Patients attending medical OPD with probable clinical diagnosis as breast cancer with/without metastasis to axillary lymph nodes, operated upon, and immediately sent for HPE in Pathology Department were included in the study.

Exclusion criteria: Benign breast lesions and patients, who were previously treated with neoadjuvant chemotherapy or radiotherapy before surgery were excluded.

Procedure

Specimens fulfilling the inclusion and exclusion criteria were sent to the Pathology Department for HPE in 10% buffered formalin. Grossing and reporting of specimens suggestive of breast carcinoma were conducted according to the CAP (College of American Pathologists) protocol [10]. Five micrometers thick sections from formalin-fixed paraffin-embedded blocks were cut and stained with Haematoxylin and Eosin (H&E) for histopathological diagnosis.

The samples which were positive for breast carcinoma by histopathology were further studied with IHC markers and the positivity of IHC expression was reported using standard procedure and scoring pattern.The Allred scoring system for hormone receptors along with American Society of Clinical Oncology (ASCO) and issued recommendations for reporting the results of HER2/neu testing was used [11,12]. The association of VEGF-C expression with respect to histological type, grade, and stage of cancer as well as the association of HER2/neu and ER/PR status was studied. Parameters studied were age of the patient, size of the breast lump, presence of lymph node metastasis, HPE using H&E staining, grading, and IHC study with VEGF-C, HER2/neu and ER/PR status. Reporting was done by trained histopathologists.

For IHC staining, 3 µm thick sections from formalin-fixed paraffin-embedded tissues were taken on poly-L-Lysine coated slides. IHC was done manually using rabbit monoclonal antibody and the steps mentioned in the kit supplied were followed. A semiquantitative assessment of VEGF-C staining was conducted [5]. Negative controls were treated identically by omitting the primary antibody. Positive control was obtained from glioblastoma tumour tissue. VEGF-C protein is found in the cytoplasm of the tumour cells. On IHC-stained slides, VEGF-C is seen as yellow-brown stains in the cytoplasm. A cytoplasmic staining pattern was considered for invasive tumour cells. VEGF-C IHC staining results were expressed as a dichotomous variable, staining <10% was classified as VEGF-C -negative, and ≥10% was classified as VEGF-C positive. The percentage of cells staining on a graduated percentage (0-100%); for positive cases, 10-30% of tumour cells in the section were positive (1+); 30-60% of tumour cells were positive (2+); 60-100% of tumour cells were positive (3+) [13].

The percentage of positive staining=(the number of positive samples/the number of samples tested) ×100%. Evaluation of paraffin IHC was performed by three reviewers. The inter-observer agreement was 90.24% with a kappa value of 0.79 signifying a substantial level of agreement.

Strong brown staining of nuclei is seen with positive ER and PR. Two parameters evaluated in IHC preparation of hormone receptors are the number of tumour cell nuclei stained and the intensity of reaction. The first is expressed as a percentage of the entire tumour cell nuclei and the following scores were given. Score for proportion: 0: None, 1: <1%, 2:1%-10%, 3: 10%-33%, 4:33%-66%, 5: 66%-100%. The intensity was graded as-0-none 1-weak, 2-moderate, and 3-strong. The scores of the two parameters were added to obtain a total score that ranges from 0-8. Patients with tumours scoring 2 or less are regarded as ER/PR negative and have a negligible chance of response to hormonal therapy.

Grading of IHC staining for HER2/neu overexpression was as [14]-

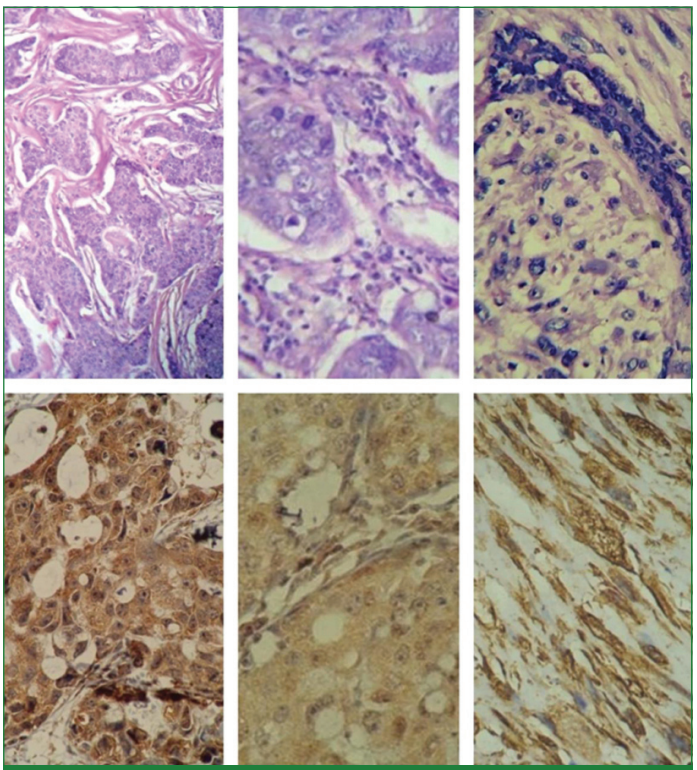
- Negative (Score 0)- No staining observed or membrane stating that is incomplete and is faint/barely perceptible and within ≤10% of tumour cells.
- Negative (Score 1+) incomplete membrane staining that is faint/barely perceptible and within >10% of tumour cells.
- Equivocal (Score 2+)- Weak to moderate complete membrane staining in >10% of tumour cells or Complete membrane staining that is intense but within ≤10% of tumour cells.
- Positive (Score 3+) Complete membrane staining that is intense and >10% of tumour cells. Both weakly positive and strongly positive cases were considered to be positive.

STATISTICAL ANALYSIS

Data were entered in MS excel and Mean±SD, range, and percentage were calculated as descriptive statistics. Using the SPSS version 18.0, Chi-square was applied for the significance of p-value and the significance level was considered at a p-value <0.05.

RESULTS

Among 44 cases, a maximum number of cases, 34 (77.27%) were infiltrating ductal carcinoma, with no specific type. One case (2.27%) was infiltrating lobular carcinoma. In the rest of the nine cases of a specific type of ductal carcinoma, 5 (11.36%) were Invasive Breast Carcinoma (IBC) of No Special Type (NST) with a medullary pattern, 2 (4.54%) were mucinous, 1 (2.27%) was metaplastic and 1 (2.27%) was papillary CA breast [Table/Fig-1].



[Table/Fig-1]: a) Infiltrating ductal carcinoma- Grade II (H&E, X100); b) Medullary carcinoma- lymphoplasmacytic infiltration; c) Metaplastic carcinoma breast; d) Infiltrating ductal carcinoma VEGF-C positivity (cytoplasmic staining) (H&E, X400); e) Medullary carcinoma- VEGF-C positivity (400x); f) Metaplastic carcinoma breast, both ductal and stromal cells are malignant Vimentin positive (cytoplasmic) stromal (H&E, X400) cells.

A total of 10 cases of Triple Negative Breast Carcinoma (TNBC) were encountered in the study which included one metaplastic carcinoma, two cases were IBC-NST with a medullary pattern and the rest seven were [Invasive Ductal Carcinoma of the breast

(IDC) NST] cases. Association with the histological stage was not done. Not all HER2/neu negative cases were PR positive. Among three, grade I cases, two were VEGF-C negative and one case was VEGF-C positive. Among 17, grade II cases, 7 cases were positive and 10 cases were negative. In 24 grade III cases, 17 were VEGF-C positive and 7 were negative. VEGF-C positivity is seen more in higher grades (grade III) than in lower grades (grade I+grade II). Among 25 VEGFC-positive cases, one case (4%) was grade I, seven cases (28%) was grade II, and 17 cases (68%) were grade III. Among 19 VEGF-C negative cases, 2 cases (10.52%) were grade I, 10 cases (52.63%) and 7 cases (36.84%) were grade I and III, respectively. There is a significant association between VEGF-C and higher grades of breast cancer (Chi-square value 4.22, p-value=0.039, p-value<0.05) [Table/Fig-2].

VEGF-C	Parameters			χ^2 value	p-value
VEGF-C	**Grade I**	**Grade II**	**Grade III**		
Positive	01 (4%)	07 (28%)	17 (68%)	4.22	0.039
Negative	02 (10.52%)	10 (52.63%)	07 (36.84%)		
Total	03 (6.82%)	17 (38.64%)	24 (54.55%)		
VEGF-C	**Nodal metastasis positive**	**Nodal metastasis negative**	**Total**	6.39	0.01
Positive	16 (64%)	09 (36%)	25 (100%)		
Negative	04 (21.05%)	15 (78.95%)	19 (100%)		
Total	20 (45.45%)	24 (54.55%)	44 (100%)		
VEGF-C	**ER Positive**	**ER Negative**	**Total**	5.23	0.022
Positive	6 (24%)	19 (76%)	25 (100%)		
Negative	11 (57.89%)	08 (42.10%)	19 (100%)		
Total	17 (38.64%)	27 (61.36%)	44 (100%)		
VEGF-C	**PR positive**	**PR negative**	**Total**	2.76	0.096
Positive	07 (28%)	18 (72%)	25 (100%)		
Negative	10 (52.63%)	09 (47.37%)	19 (100%)		
Total	17 (38.64%)	27 (61.36%)	44 (100%)		
VEGF-C	**HER2/neu Positive**	**HER2/neu Negative**	**Total**	8.84	0.003
Positive	19 (76%)	06 (24%)	25 (100%)		
Negative	05 (26.32%)	14 (73.68%)	19 (100%)		
Total	24 (45.45%)	20 (54.55%)	44 (100%)		

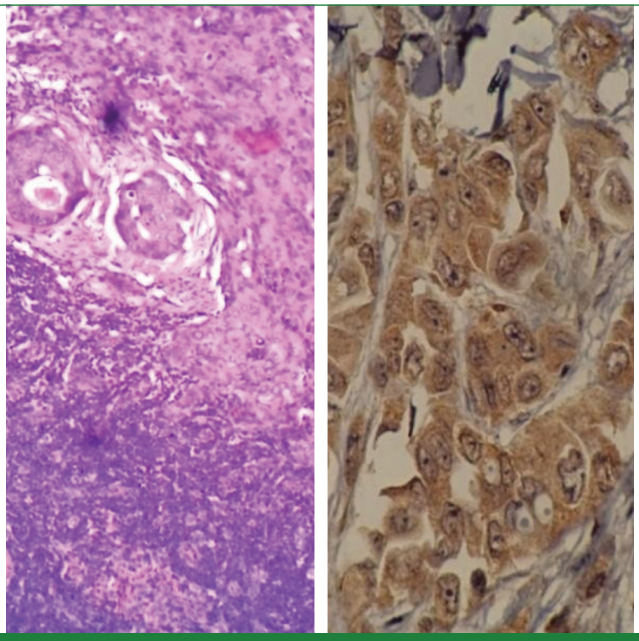
[Table/Fig-2]: Parameters data in tabular form. Association of VEGF-C expression with tumour grade, presence of nodal metastasis, ER, PR and HER2/neu status along with statistical analysis.

Among 25, VEGF-C positive cases, 16 (64%) were metastatic and 9 (36%) cases were non metastatic [Table/Fig-2,3]. Among 19, VEGF-C negative cases, 4 (21.05%) cases were metastatic and 15 (78.95%) cases were non metastatic to axillary lymph nodes. There was a significant association between VEGF-C and axillary lymph node metastasis of breast cancer {Chi-square value (with Yates correction) 6.39, p-value=0.01, p-value <0.05 [Table/Fig-2,3]}.

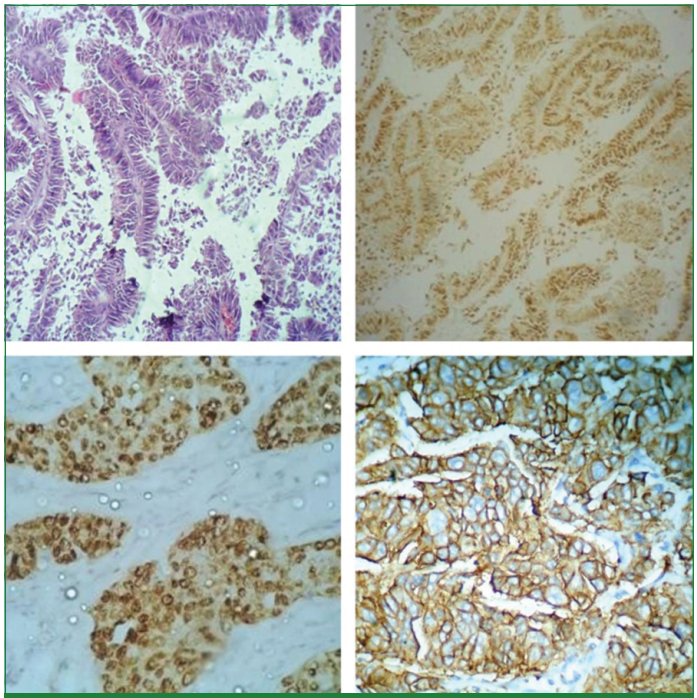
Among 25, VEGF-C positive cases, 6 (24%) were ER-positive and 19 (76%) cases were ER-negative. Among 19, VEGF-C negative cases, 11 (57.89%) cases were ER positive and 8 (42.10%) cases were ER negative. There is a significant association between VEGF-C and ER status of breast carcinoma. (Chi-square value 5.23, p-value=0.022, p-value<0.05) [Table/Fig-2,4].

Among 25 VEGF-C positive cases, 7 (28%) cases were PR positive and 18 (72%) were PR negative. Among 19 VEGF-C negative cases, 10 (52.63%) were PR positive and 09 (47.37%) were PR negative. There is no significant association between PR status and VEGF-C positivity. (Chi-square value 2.76, p-value=0.096, p-value>0.05) [Table/Fig-2].

Among 25 VEGF-C positive cases, 19 (76%) were HER2/neu positive and 06 (24%) were HER2/neu negative. Among 19 VEGF-C negative cases, 05 (26.32%) were HER2/neu positive and 14 (73.68%) were HER2/neu negative. There is a significant association between HER2/neu status and VEGF-C positivity. Chi-square value (with Yates correction) 8.84, p-value<0.05 [Table/Fig-2,4]. A significant positive association was seen between VEGF-C positivity and HER2/neu status (Chi-square value, 8.84, p-value=0.003, p-value<0.01).



[Table/Fig-3]: a) Left panel -Lymph node metastatic deposit of infiltrating ductal carcinoma of breast (H&E stain 400X); b) Right panel- Metastatic deposit of infiltrating ductal carcinoma showing VEGF-C positivity (cytoplasmic staining) (400X).



[Table/Fig-4]: a) Upper left panel- Infiltrating papillary carcinoma- (H&E 100X); b) Upper right panel- Infiltrating papillary carcinoma- with PR positivity (100X); c) Lower left panel-Infiltrating ductal carcinoma with ER positivity (400X); d) Lower right panel- Infiltrating ductal carcinoma HER2/neu positivity (400X).

DISCUSSION

After IHC staining, 25 (56.82%) were VEGF-C positive and the rest i.e., 43.18% were VEGF-C negative. Among 25, VEGF-C positive cases of breast carcinoma, 4% are in grade I, 28% are in grade II and 68% are in grade III. VEGF-C positivity differs significantly in grades of breast carcinoma (Chi-square value=4.22, p-value<0.05) and there was a significant positive association between VEGF-C positivity and higher grades of breast carcinoma (Chi-Square value 4.22, p-value=0.039, p-value<0.05). A study conducted by Hoar FJ et al., showed there was no significant association between

VEGF-C and grades of tumour [15]. He studied 51 cases, out of which 59% were positive for VEGF-C. He found no significant association between VEGF-C expression and lymph node status, LVI, tumour size, grade, or ER status.

Another study by Guo XJ et al., demonstrated that VEGF-C positivity is seen more in higher grades (II/III) of breast carcinoma, invasive micropapillary type than in grade I (p-value=0.03) [16]. Among 25 VEGF-C positive cases of studied breast carcinoma, 64% were metastatic to any group of axillary lymph nodes and 36% were non metastatic. In 19 VEGF-C negative cases, only 21.05% were metastatic and the rest 78.95% were non metastatic. A significant association was found between VEGF-C and nodal metastasis in the present study.

A study by Skobe M et al., showed that the degree of tumour lymphangiogenesis had high concordance with the extent of lymph node and lung metastases, and they established the occurrence and biological significance of intratumoural lymphangiogenesis in breast cancer and identified VEGF-C as a molecular link between tumour lymphangiogenesis and metastasis [17].

In 2003 Nakamura Y et al., studied 123 cases of invasive breast carcinoma and also showed that VEGF-C positivity was significantly associated with lymph node metastasis (p-value=0.0131) [18]. In 2010 Schoppmann SF et al., found that VEGF-C correlates significantly with LVI and lymphatic microvessel density [19]. Guo XJ et al., showed that there was a significant association between VEGF-C and lymph node metastasis (p-value=0.006) and lymphatic vessel density (p-value=0.009) [16]. Another study by Ran S et al., showed that increased VEGF-C positivity is associated with increased lymph nodal metastasis [20]. But in 2003, Hoar FJ et al., found no significant association between VEGF-C and lymph nodal status and LVI [15].

VEGF-C seems to be negatively associated with ER status of breast carcinoma and was not associated with the PR status of breast carcinoma. Among 25 VEGF-C positive cases, only 24% were ER positive and the rest (76%) were ER negative. Similarly, among 19 VEGF-C negative cases, 57.89% were ER positive and 42.10% were ER negative. There was a significant association between VEGF-C and ER status of breast carcinoma (Chi-square value=5.23 and p-value<0.05) [21]. Hoar FJ et al., demonstrated that there was no significant association between VEGF-C and ER status [15]. In 2007, Al Mowallad A et al., failed to demonstrate any association between VEGF-C and ER status [22].

In cases with VEGF-C positive staining, 28% were PR positive and 72% were PR negative. Among 19 VEGF-C negative stainings, 52.63% were PR positive and 47.37% were PR negative. Similar to our study findings, Yavuz S et al., and Al Mowallad A et al., also could not find any association between VEGF-C and PR status in breast carcinoma [7,22].

Authors hereby, found that VEGF-C was significantly associated with lymph node metastasis and is positively associated with HER2/neu. In this study, among 25 VEGF-C positive cases, we found 76% HER2/neu positive and 24% HER2/neu negative cases. Similarly, in 19 VEGF-C negative cases, there were 73.68% HER2/neu negative and 26.32% HER2/neu positive cases. There was a significant association between VEGF-C and HER2/neu (Chi-square value=8.84, p-value=0.003, p-value<0.01). Also, there was a significant positive association between VEGF-C and HER2/neu status of breast carcinoma. In 2002 Yang W et al., demonstrated that the expressions of VEGF-C were significantly and positively associated with Erythroblastic oncogene B receptor 2 (ERBB2) expression [8]. In 2004 Konecny GE et al., demonstrated that there is a significant positive association between HER2/neu and VEGF-C levels (p-value<0.05). There is poor outcome among patients with tumours demonstrating both HER2/neu overexpression and VEGF-C expression and favourable outcome for patients whose tumours had both normal HER2/neu expression and no detectable VEGF-C expression [23].

Another study by Schoppmann SF et al., showed that there was a clinically relevant association between HER2/neu and VEGF-C expression in human breast cancer. They proposed that by inhibiting HER2/neu, tumour progression may be reduced by blocking VEGF-C-mediated tumour cell proliferation and lymph node metastasis. HER2/neu strong positivity (3+) showed a significantly stronger VEGF-C expression than all other cases (p-value=0.006). They also showed a significant correlation between VEGF-C expression and LMVD (p-value=0.012) and a strong positive association between LMVD and LVI (p-value=0.001) [19].

In 2003 Hoar FJ et al., demonstrated that there was an association between Humanized epidermal growth factor receptor 2 (c-ERBB2) and VEGF-C expression (p-value=0.013). They also proposed that the association between VEGF-C and c-erbB2 is suggestive of a functional relationship between these and may, in part, explain the aggressive phenotype associated with c-erbB2-positive tumours [15]. In a study by Fu JM in 2009 showed that the expression of VEGF-C was positively associated to that of C-erbB 2 (p-value<0.05) [21].

Limitation(s)

Present study is limited by small sample size. Further study with larger samples is required for more accurate findings.

CONCLUSION(S)

According to latest treatment guidelines in patients of breast cancer adjuvant therapy is required in 90% of node negative cases. Despite the fact that only 30% of cases are benefited with this treatment. Thus, it is need of an hour to identify a new marker for these subsets of patients who then may not need adjuvant chemotherapy. In this regard, assessment of VEGF-C is recommended because expression of VEGF-C is one of the factors determining the aggressiveness of breast carcinoma. If VEGF-C is positive in node-negative breast carcinoma, then it may help in targeted therapy of breast carcinoma before lymphatic metastasis develops.

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