



Study on Carcinomas of Breast with Special Reference to Expression of Vascular Endothelial Growth Factor and Her 2/neu

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i20B31360

Editor(s):

(1) Dr. Ana Cláudia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.

Reviewers:

(1) Athiraja A, Rathinam Technical Campus, India.

(2) Priya J. P. Narayan, Swami Rama Himalayan University, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/66758>

Original Research Article

Received 27 January 2021

Accepted 02 April 2021

Published 06 April 2021

ABSTRACT

Carcinoma is a major health issue worldwide. Breast cancer is by far the most common of all cancers diagnosed in women, in the world. The incidence of breast cancer has risen in India (22.9%). Breast cancer cases in India are expected to increase by 26% by 2020. There be existing 2,088,849 new cases of breast cancer worldwide foremost to 626,679 deaths in 2018. Among the Indian women, cancer cervix and breast carcinoma account for 60% of the total cases, of which the incidence of breast carcinoma is 10.4%. Angiogenesis is nothing but the growth of new vasculature from the pre-existing vasculature. This physiological process is involved in organ and embryonic development, wound healing, and reproductive functions in the adults. However, pathologically, angiogenesis is implicated in macular degeneration, rheumatoid arthritis, psoriasis, and tumour growth. HER2/neu status has recently become clinically very relevant because it has been demonstrated that HER-2/neu positive tumors have worse prognosis than HER2/neu negative tumors. To correlate the expressions of the Immunohistochemical markers HER-2/neu and Vascular Endothelial Growth factor (VEGF) with the various clinicopathological parameters like age, tumour size, histological type, grade, stage and lymph node metastasis status. It concludes an unequivocal association between the expressions of the two immunohistochemical markers, HER-2/neu and VEGF in breast carcinoma cases and its implication in guiding therapy against HER-2/neu over-expressing tumors.

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Keywords: Carcinoma; breast cancer; HER-2/neu; VEGF.

1. INTRODUCTION

Breast cancer is a heterogeneous disease. It is a major health problem and a leading cause of death among women. Breast cancer causes around 3,76,000 deaths per year, globally and almost 9,00,000 new cases are diagnosed every year [1-5]. Among the Indian women, carcinoma of the breast and cervix, together account for around 60% of total cases, of which breast carcinoma accounts for 10.4% [4]. The mean age of occurrence is 42 years [5,6]. Although communicable diseases can be implicated as a major cause of mortality, non-communicable diseases (including cancer) now account for more than 50% of deaths in India. Although overall cancer incidence is lower in India than in most of the developed countries, the relative mortality rates are higher [7].

The approach to the management of breast carcinoma has undergone tremendous changes over the last 20 years. Today, the choice of treatment is conservative and reconstructive surgery than mastectomy, in most of the cases. These changes have been accompanied by increasing use of systemic, hormonal and cytotoxic drugs, which are used in both adjuvant and neoadjuvant settings. Prognosis and management of breast carcinoma depends on few classical variables, such as histological type, grade, tumour size, lymph node status, hormonal status etc. Identification of biomarkers also plays an essential role in the treatment, management and prognosis of breast carcinoma [8,9].

HER2/neu status has recently become clinically very relevant because it has been demonstrated that HER-2/neu positive tumours have worse prognosis than HER2/neu negative tumors. It has been recognised that HER2/neu over-expression served both as a marker of aggressive disease and a target for treatment. HER2/neu status not only predicts poor outcome, but also the sensitivity to treatment, with drugs like Trastuzumab (Herceptin), which is a humanized monoclonal anti-HER2/neu antibody. With these prognostic implications, the need for accurate and precise assessment of HER2/neu expression in breast carcinoma becomes critical, in the determination of patients who will benefit from treatment with the above mentioned drugs [10,11,12].

Angiogenesis is nothing but the growth of new vasculature from the pre-existing vasculature.

This physiological process is involved in organ and embryonic development, wound healing, and reproductive functions in the adults. However, pathologically, angiogenesis is implicated in macular degeneration, rheumatoid arthritis, psoriasis, and tumour growth. Many clinicopathological studies have confirmed about the central role of angiogenesis in breast cancer progression. Hypoxia is the main signal for the induction of angiogenesis. Multiple angiogenic factors are commonly expressed by invasive human breast cancers. At least, six different pro-angiogenic factors have been identified with the Vascular endothelial growth factor predominating. The role of VEGF in breast cancer is not only limited to angiogenesis, the cancer cells may be able to promote their own growth and avoid apoptosis through VEGF [13,14,15].

Numerous studies have implicated that activated HER2/neu potentiates tumour cell adhesion to endothelial cells, which leads to an increase in hypoxia-inducible factor 1 alpha-mediated VEGF A expression and facilitates angiogenesis and vascular invasion. Hence, finding out the association between HER2/neu and VEGF expressions in breast carcinoma, helps in predicting the prognosis and determining the therapy which will be effective for the patients. And to find out this association, is the central theme of this study.

2. MATERIALS AND METHODS

The present study was conducted in the department of Pathology, Sree Balaji Medical College and Hospital, from November 2016 to October 2018. This study included the cases of carcinoma breast, confirmed by biopsy.

2.1 Inclusion and Exclusion Criteria

Modified radical mastectomy and lumpectomy specimens with axillary clearance (30 in number), which were received in our department, were included in the study.

2.2 Methods

Processing of specimen and fixation was done in 10% buffered formalin. Grossing was done as per the guidelines of National Cancer Institute, standardized management of breast specimens recommended by Pathology Working group, Breast cancer Task ForceP [16]. Four to five micrometer thick, formalin fixed, paraffin

embedded tumour sections were stained with Haematoxylin and Eosin. Histologic examination to find out the tumour type and grading was performed routinely according to the criteria outlined in the World Health Organization classification of tumors [16].

The microscopic grading of breast carcinomas was done as per the Nottingham Modification of Bloom-Richardson-grading system.. The staging was done in accordance with the American joint committee on cancer (AJCC) staging system which includes both clinical and pathological staging, based on TNM characteristics. T stands for tumour size, N for nodes and M denotes metastasis .

2.3 Immunohistochemical Analysis

Immunohistochemical evaluation of HER2 and VEGF was done on poly-L lysine coated slides by using polymer two step indirect method. The antibodies and chemicals were obtained from Biogenex, USA. The anti-VEGF (clone 165) and anti-human C-erb-B-2 (clone CB11) monoclonal antibodies were used for Immunohistochemical evaluation of VEGF and Her-2 respectively.

3. RESULTS

During the study period, from November 2016 to October 2018, 30 cases of carcinoma breast, which were confirmed by histopathology, were included in this study.

3.1 Clinicopathologic Profile of the Cases

Among the 30 studied cases, 40%, 40% and 20% belonged to the age-groups of 35-45 years, 45-55 years and >55 years, respectively (Table 1 & Chart 1). The mean age of patients in this study group was 47.5 years. Only female breast cancer cases were included in the present study.

The T2 lesions (ranging between the size of 2-5 cm) accounted for 70% of cases, while T3 (>5 cm in size) and T1 lesions (<2 cm in size) accounted for 26.7% and 3.3% of cases, respectively. (Table 2 & Chart 2).

Out of the total 30 studied cases, Invasive carcinoma of no special type (Fig. 1), was the commonest histologic type accounting for 80% (24/30) cases. There were two cases each of Invasive lobular carcinoma (Fig. 4) and mucinous carcinoma (Fig. 5) and one case each of metaplastic carcinoma of no special type (Fig. 7) and invasive papillary carcinoma (Fig. 9). (Table 3 and Chart 3).

Regarding the histologic grades of the tumours, in this study 13.3% were grade I, 40% belonged to grade II and 46.7% were grade III tumours (Table 4) Since grade I tumours were very few in numbers, grade I and grade II tumours could also be clubbed together as low grade tumours and grade III were treated as high grade tumors Hence, low grade tumours made up 53.3% (16 out of 30) whereas high grade tumours made up 46.7% (14 out of 30) of the 30 cases, which were included in this study.

60% of the cases included in this study showed AJCC stage II tumours and 40% of the cases showed AJCC stage III tumors none of the tumors belonged to stage I and stage IV. (Table 5).

Lymph node metastasis was seen in 63.3% cases while 36.7% cases were negative for it (Table 6).

Out of 30 cases, HER2/neu positivity was found in 50% cases and the other 50% were negative for this immunohistochemical marker. (Table 7).

VEGF positivity was seen in 70% of the breast tumours, while 30% showed negativity for it. (Table 8).

Association between histologic type and HER2/neu expression is depicted in Table 7. Out of 30 cases, 24 (80%) were Invasive carcinoma no special type and 2 (6.7%) cases each of invasive lobular carcinoma

Table 1. Age wise distribution of the tumours

Age groups	Percentage	Frequency
35-45	40.0%	12
45-55	40.0%	12
>=55	20.0%	6
Total	100.0%	30

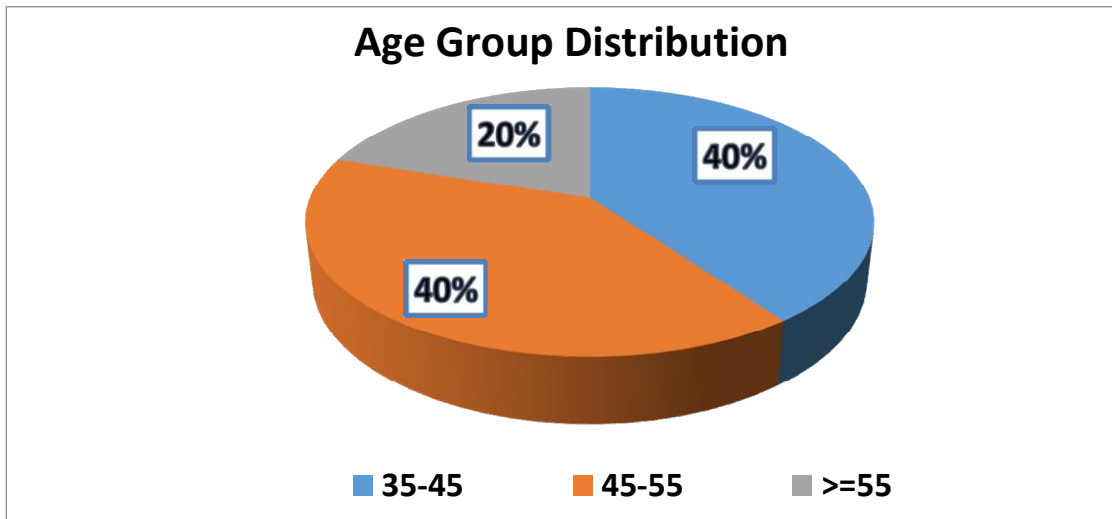


Chart 1. Age wise distribution of the tumours

Table 2. Size wise distribution of the tumours

Tumour Size	Percentage	Frequency
T1 (<2 cm)	3.3%	1
T2 (2-5 cm)	70.0%	21
T3 (>5 cm)	26.7%	8
Total	100.0%	30

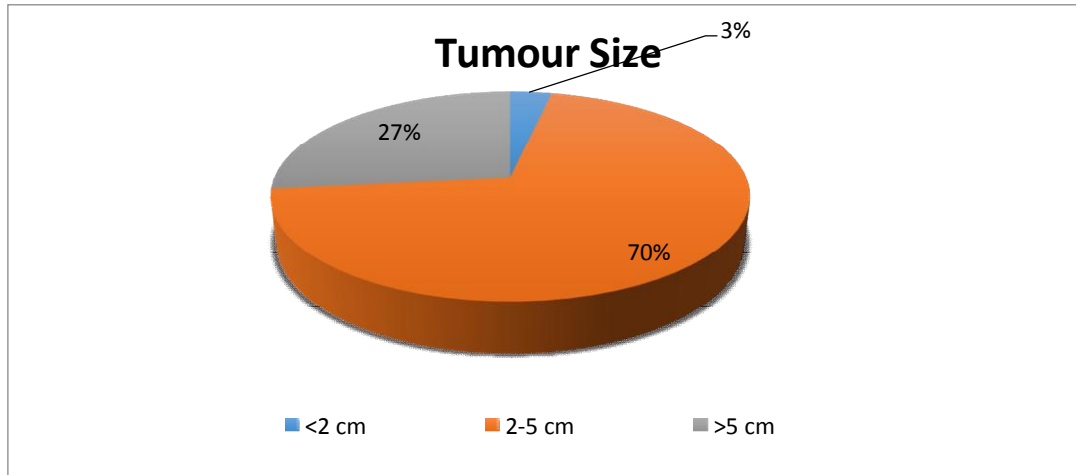


Chart 2. Size wise distribution of the tumours

Table 3. Distribution of cases according to histological diagnosis

Histological Diagnosis	Percentage	Frequency
Invasive carcinoma of no special type	80.0%	24
Invasive Lobular Carcinoma	6.7%	2
Mucinous Carcinoma	6.7%	2
Metaplastic	3.3%	1
Carcinoma of No Special Type		
Invasive Papillary Carcinoma	3.3%	1
Total	100.0%	30

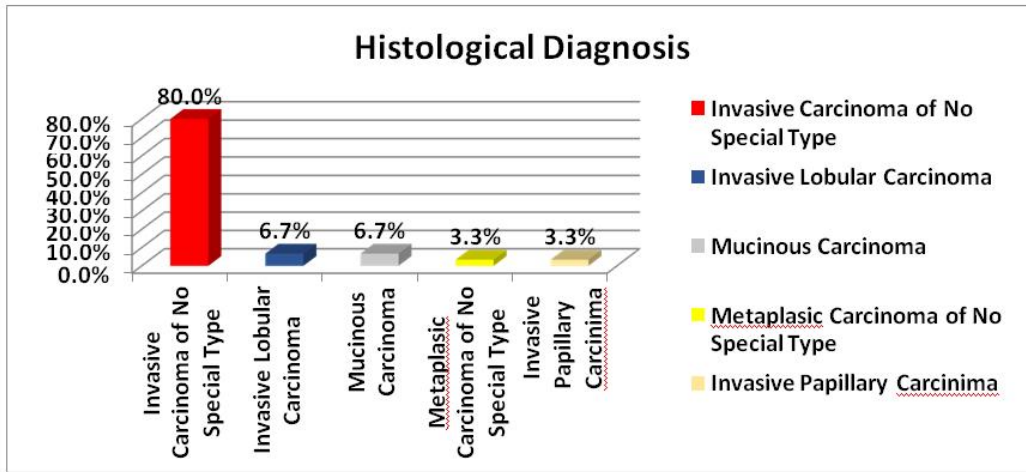


Chart 3. Distribution of cases according to histological diagnosis

Table 4. Histological grade wise distribution of tumours

Histological Grading	Percentage	Frequency
I	13.3%	4
II	40.0%	12
III	46.7%	14
Total	100.0%	30

Table 5. Histological stage wise distribution of the tumors

Staging	Percentage	Frequency
II	60.0%	18
III	40.0%	12
Total	100.0%	30

Table 6. Distribution of tumours on the basis of lymph node metastasis

Lymph node metastasis	Percentage	Frequency
Absent	36.7%	11
Present	63.3%	19
Total	100.0%	30

Table 7. Distribution of expression of HER2/neu in Breast Carcinoma cases

Human epidermal growth factor receptor 2 expression	Percentage	Frequency
Negative	50.0%	15
Positive	50.0%	15
Total	100.0%	30

Table 8. Distribution of expression of VEGF in breast carcinoma cases

Vascular Endothelial Growth Factor Expression	Percentage	Frequency
Negative	30.0%	9
Positive	70.0%	21
Total	100.0%	30

and mucinous carcinoma. There were 1 (3.3%) case, each of metaplastic carcinoma of no special type and invasive papillary carcinoma. Out of the 24 cases of Invasive carcinoma of no special type, 15 cases were HER2/neu positive (Fig. 2) while 9 cases were negative. This makes us conclude that majority of the cases of invasive ductal carcinoma of no special type are positive for Her 2/neu. None of the other histological types showed HER2/neu positivity. Taking into consideration the small numbers of cases of invasive lobular carcinoma, mucinous carcinoma, metaplastic carcinoma of no special type and invasive papillary carcinoma, the presence or absence of association of HER2/neu expression.

Association between histologic type and VEGF expression has been presented in Table 8. Out of 30 cases, 24 were Invasive carcinoma of no special type, 2 each belonged to invasive lobular carcinoma and mucinous carcinoma, with 1 case each of metaplastic carcinoma of no special type and invasive papillary carcinoma. The composition revealed the common manifestation of Invasive carcinoma of no special type and rare occurrence of the other histological types of the tumors 75% (18 out of 24) of the cases of Invasive carcinoma of no special type were

VEGF positive (Fig. 3) while both the cases of invasive lobular carcinoma are VEGF negative. 1 out of 2 (50%) cases of mucinous carcinoma was VEGF positive (Fig. 6) and the other 1 was negative. 1 (100%) case, each of Metaplastic carcinoma of no special type and invasive papillary carcinoma was VEGF positive (Fig. 8 and Fig. 10). On the basis of these findings, we can say with definiteness that Invasive carcinoma of no special type has a strong association with VEGF positivity. However, nothing much can be commented about the association of VEGF expression with the other histological types, because of the very few number of cases of these entities.

4. DISCUSSION

Breast cancer is the most common cancer among the females, all over the world. The global burden of breast cancer has been estimated to increase to 2 million new cases per year, by year 2030 [16]. It can occur at any age but is rarely observed in patients younger than 25 years and over 80 years [12,17].

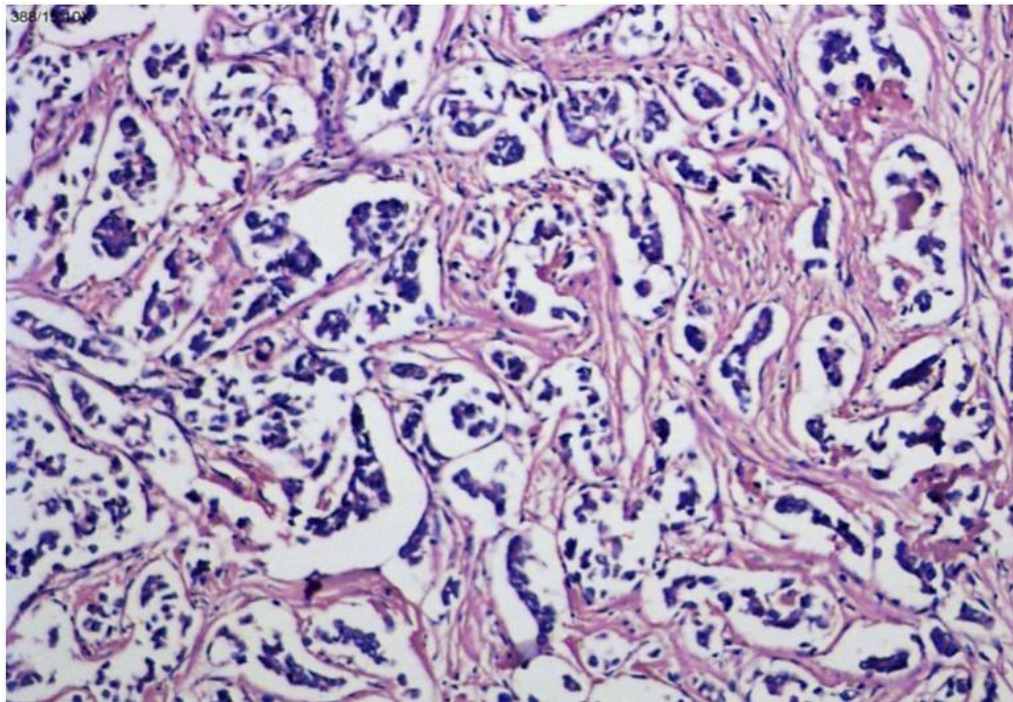


Fig. 1. Invasive breast carcinoma of no special type (H & E, 10X)

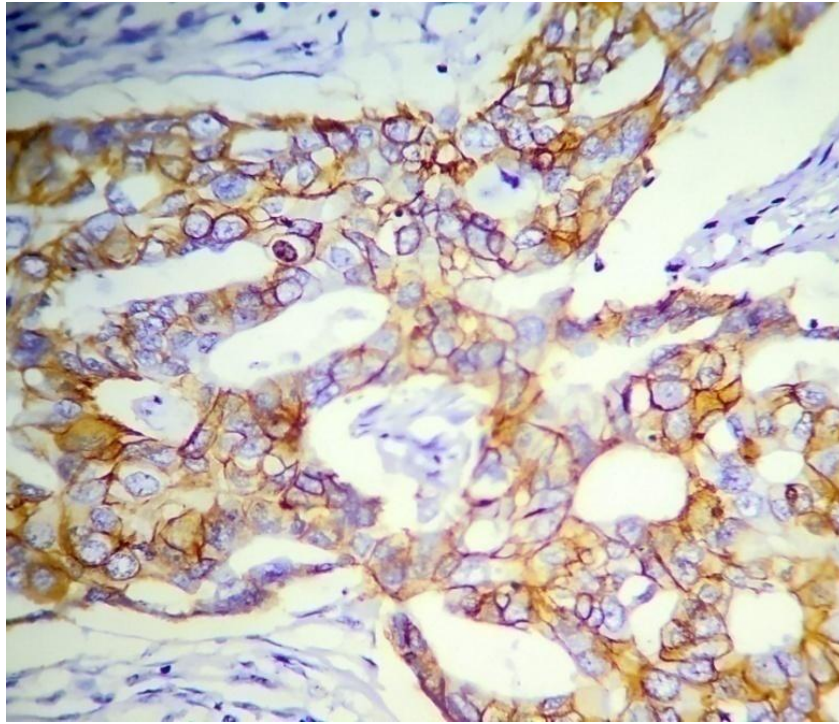


Fig. 2. Invasive breast carcinoma of no special type, showing positivity for HER2/neu (HER2/neu, 40X)

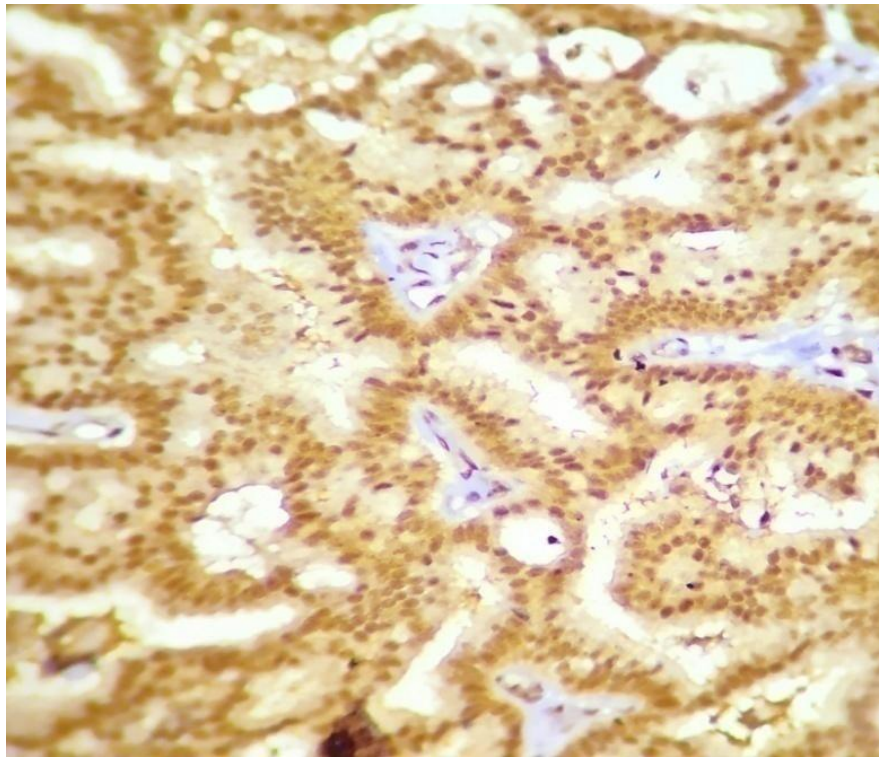


Fig. 3. Invasive breast carcinoma of no special type, showing positivity for VEGF (VEGF, 40X)

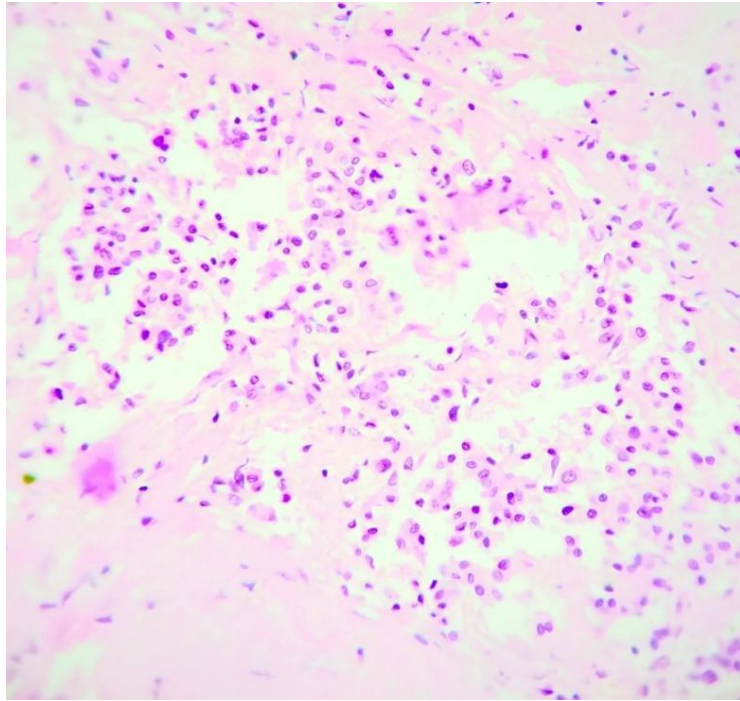


Fig. 4. Invasive lobular carcinoma (H & E, 10X)

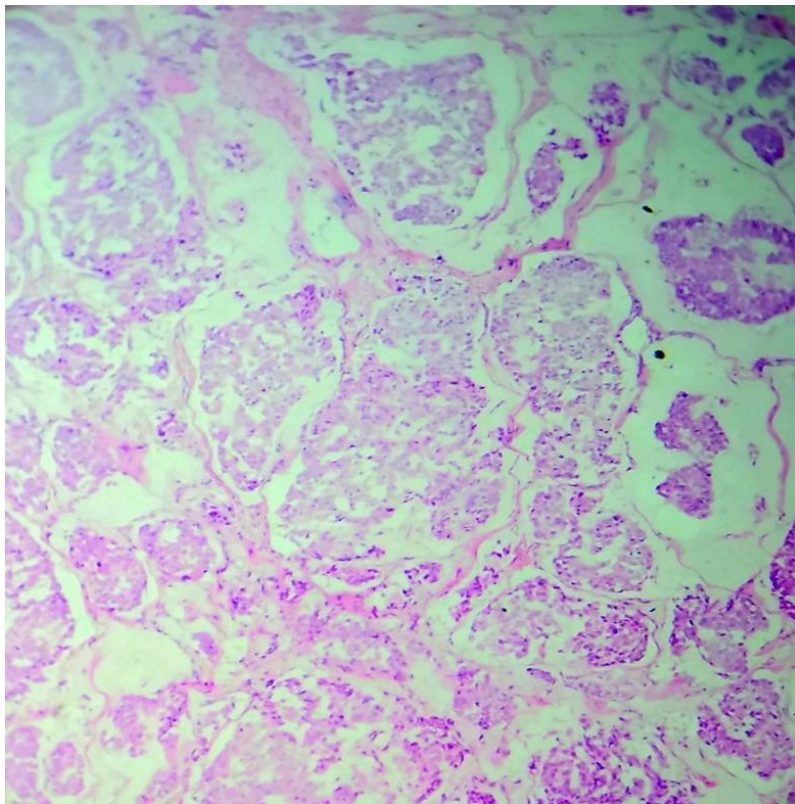


Fig. 5. Mucinous carcinoma (H & E, 10X)

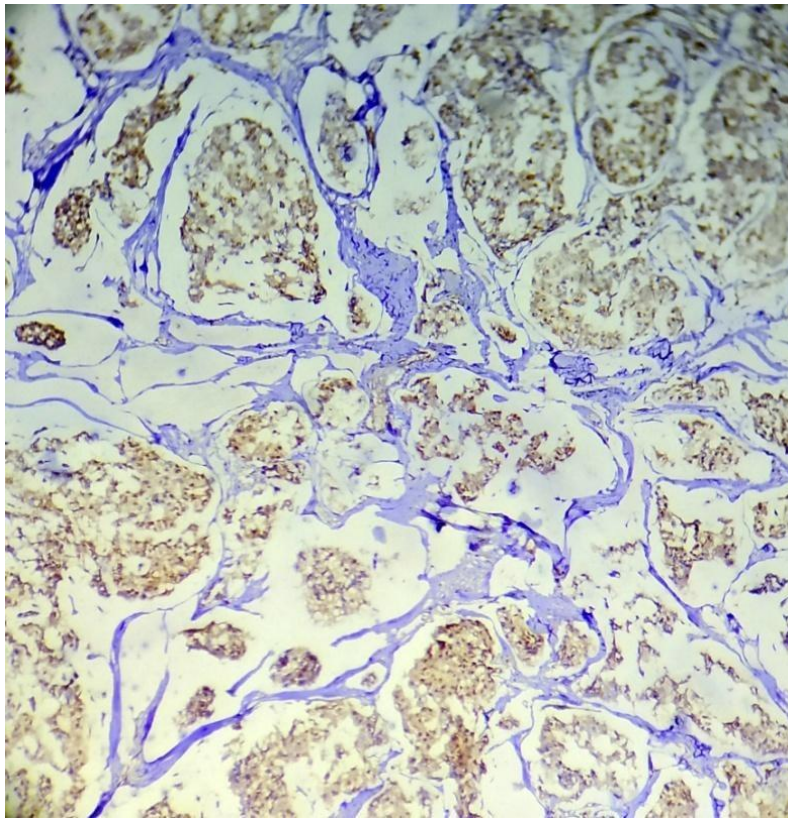


Fig. 6. Mucinous carcinoma, showing VEGF positivity (VEGF, 10X)

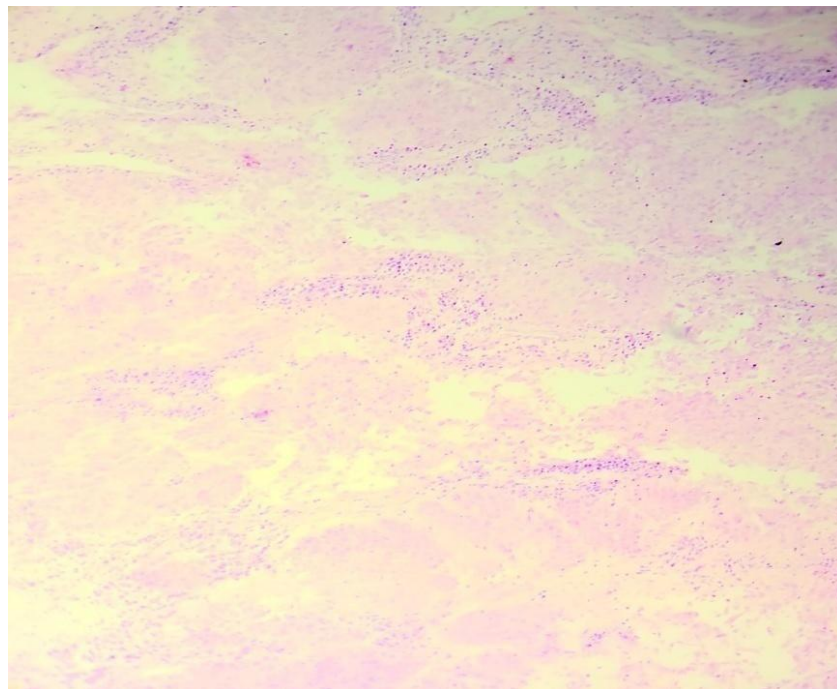


Fig. 7. Metaplastic carcinoma of no special type (H & E, 10X)

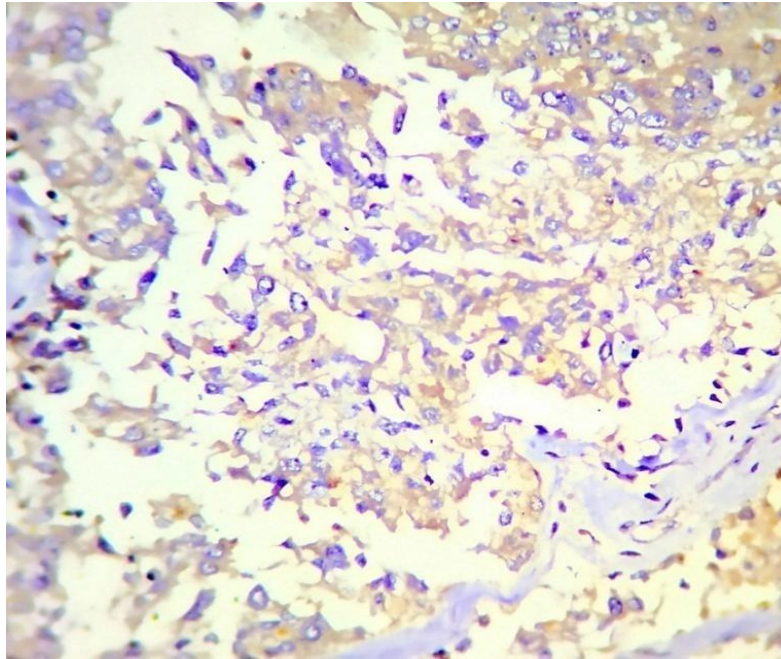


Fig. 8. Metaplastic carcinoma of no special type, showing VEGF positivity (VEGF, 40X)

Now, histological and molecular analysis have demonstrated that breast cancer is a heterogeneous disease, composed of morphologically and genetically distinct entities with different molecular profiles, behaviour, and response to therapy. At morphologic level, breast cancer is classified according to the histological types and grades. At molecular level, breast cancer has been classified according to hormone receptor status and HER2/neu status. New molecular taxonomies have identified distinct sub- types like luminal, HER2/neu positive and basal-like classes [17].

The up-regulation of HER-2 is associated with increased expression of vascular endothelial growth factor at both the RNA and protein levels in breast carcinoma cells and exposure of HER-2-positive cells to Trastuzumab significantly decreases VEGF expression. Src, a downstream adaptor protein of the HER-2 signalling pathway, has been found to be a switch for VEGF production showing that VEGF is a downstream target of the HER-2 signalling pathway. The combination treatment with HER- 2/neu and mimics of VEGF peptide together, produce additive effects. This explains that targeting the two different receptors, brings about greater anti-tumour and anti-angiogenic effects both in vitro and in vivo [18-20].

This study was designed with the primary objective of finding an association between the immunohistochemical expressions of HER2/neu and VEGF in breast carcinoma cases, which is important because VEGF has been implicated in aggressive phenotype of breast cancers that over- express HER2/neu. The reaffirmation of this relationship would support the use of combination therapies directed against both HER2/neu and VEGF for treatment of the cases that show HER2/neu over-expression. The secondary objectives were to correlate the immunohistochemical expressions of the two markers to the various clinico-pathological parameters [21].

4.1 Association of HER2/neu and VEGF with Age

The mean age of patients in our study group was observed to be 47.5 years which was comparable to the mean age of patients in the studies conducted by Xiaowei Ye et al (49.86 years), En-Qi Qiao et al (51 years) and Wentao Yang et al (51.9 years). The mean age in the study conducted by Konecny et al (58years) was however significantly higher than that in the present study. 63.3% of patients in our study were ≤ 50 years while 36.7% of patients were >50 years compared to 49% (<50 years) and 51% (>50 years) and 51% (< 50 years) and 49 %

(>50 years) of patients in the study conducted by En-Qi Qiao et al and Xiaowei Ye et al respectively [22,23].

The present study showed no significant association between HER2/neu positivity and age. This finding was in agreement with the finding of Xiaowei Ye et al, in which they did not find any significant association between HER2/neu expression and age of the patient. Our study also revealed that the percentage of VEGF positivity has no association with age, being 73.7% in the age group ≤ 50 years and 63.6% in the age group of >50 years. This finding is in accordance with the study conducted by Xiaowei Ye et al which had also showed no association between VEGF expression and age [24].

4.2 Tumour Size

As far as the tumour size is concerned, T2 lesions (2-5 cm in size) were the most common (70%) in our study. This finding was in agreement with the studies conducted by Wentao Yang et al (62.6% of T2 lesions) and Konecny et al (51% of T2 lesions). However, T1 lesions were found to be in majority, in the works of En-Qi Qiao et al (56%), Xiaowei Ye et al (42%) and Linderholm et al (57%).

An attempt was made to find an association between VEGF and HER2/neu expressions with tumour size. There was no statistically significant association between HER2/neu expression and tumour size. These findings were similar to the observed findings of Linderholm et al and Xiaowei Ye et al. Similarly, the present study revealed no association between the expression of VEGF and tumour size. These findings were in accordance with the findings of Xiaowei Ye et al. But the proportion of difference is not very significant. Hence no significant association could be made out. These findings were in accordance with the studies of Linderholm et al, En Qi Qiao et al and Xiaowei Ye et al. None of these studies showed any significant association of HER2/neu expression with histologic grades. In all the three histologic grades, VEGF positivity was found to be highly prevalent, in our study. Therefore, the association between histologic grades and VEGF expression cannot be confirmed. Similar findings were depicted in the studies of Xiaowei Ye et al and En-Qi Qiao et al. Although Konecny et al had found a significant association between the histological grades and VEGF [25].

4.3 AJCC Staging

In this study, an attempt was made to study the association of HER2/neu and VEGF expressions with AJCC staging. This study included 60% cases of AJCC stage II and 40% cases of AJCC stage III tumors. In stage II and III tumours, the positivity of HER2/neu was of the order of 55.5% and 41.7%, respectively. The VEGF positivity was higher in AJCC stage III (75%) than in AJCC stage II (66.7%). But in both the cases, the difference in positivity were not significant enough. However, both these findings are also limited by the fact that this study does not include any AJCC stage I or AJCC stage IV tumors. Only a study with adequate numbers of cases in Stage I and Stage IV can lead to a valid conclusion, in this area [26,17,22].

4.4 Lymph Node Status

In our study, 63.3% cases were node positive which is comparable to the findings of Wentao Yang et al (61.7% node positive cases). The studies belonging to Xiaowei Ye et al, Linderholm et al, Konecny et al and En-Qi Qiao et al had 34%, 42%, 51.7% and 82% of node positive cases, respectively.

It also showed significant association between HER2/neu positivity and positive lymph node status as HER2/neu positivity was observed in 63.2% lymph node positive cases, while it was present in 27.3% of lymph node negative cases. This is in agreement to the findings of Xiaowei Ye et al, which showed a significant association between HER2/neu expression and lymph node status but against the findings of En Qi Qiao et al and Linderholm et al, as they have reported no association between the two entities. This study revealed that VEGF expression had no significant association with lymph nodes positivity or negativity as 54.5% of lymph node positive cases and 78.9% of lymph node negative cases exhibited VEGF positivity. This finding was parallel to the findings of En-Qi Qiao et al and Konecny et al. However, Xiaowei Ye et al has documented an association between lymph node positivity and VEGF expression [23,26].

4.5 Association between HER2/neu and VEGF Expressions

The HER2/neu over-expression is significantly and positively correlated to higher expression of VEGF in breast carcinoma, suggesting that VEGF may in part mediate the aggressive

phenotype of breast carcinoma that over-expresses HER2/neu. The scope of use of combination therapies against both HER2/neu and VEGF in HER2/neu over-expressing breast carcinoma cases, by proving the association between HER2/neu and VEGF expressions, was the central theme of this study. The results of study of association between these two molecular markers, lead to a indication that HER2/neu positivity has a strong association with VEGF positivity (100%). Chi square test of independence also reveals a significant association between the two factors ($p = 0.014$). Also majority of HER2/neu negative cases were correlated with negative VEGF expression (60%) [19,18].

5. CONCLUSION

The current study provides clinical evidence that HER-2/neu over- expression is associated with the over-expression of VEGF in breast carcinoma, suggesting that VEGF may in part mediate the aggressive phenotype of breast carcinoma that overexpresses HER-2/neu. These data additionally support the use of combination therapies directed against both HER-2/neu and VEGF for the treatment of breast carcinoma patients who exhibit HER-2/neu over-expression. It concludes an unequivocal association between the expressions of the two immunohistochemical markers, HER-2/neu and VEGF in breast carcinoma cases and its implication in guiding therapy against HER-2/neu over-expressing tumors.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study was approved by the Ethical Committee of the Institution.

ACKNOWLEDGEMENTS

The encouragement and support from Bharath University, Chennai is gratefully acknowledged. For provided the laboratory facilities to carry out the research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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