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Original Research Article

Expression of vimentin in breast carcinoma and its correlation with histopathological parameters

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ABSTRACT

Background and Objectives: Despite various treatment and diagnostic options, Breast cancer remains one of the top cancer in women both in developed and the developing world. In such a scenario, Immunohistochemistry plays an important role in pathology of breast disease which contributes for solving diagnostic problems and for determining prognosis and response to therapy. There is growing list of IHC markers available, the present study highlights the importance of vimentin a mesenchymal marker in breast carcinoma cases.

Objectives of the Study: 1) To assess the expression of vimentin in various types of breast carcinomas. 2) To find out the correlation between expression of vimentin and prognostic markers such as tumor size, tumor grade and lymph node status.

Materials and Methods: The study included 50 cases of modified radical mastectomy specimens received in the Department of Pathology, during December 2017 to May 2019. After collecting the history, the specimens were examined, fixed in 10% formalin and sections were studied by hematoxylin and eosin and immunohistochemically.

Results: In the present study majority of the patients were in the age group of <40 years (Mean age-51 years). Vimentin expression was seen in 23/50 (46%) of cases. Its expression was correlated significantly with high grade tumors (P value<0.05) indicating epithelial to mesenchyamal transition of tumor cells. Majority of the tumors were of grade 2(48%) and diagnosed histopathologically as invasive carcinoma breast NST. Vimentin expression did not correlate with lymph node status and tumor size (P value<0.05).

Interpretation and Conclusion: Vimentin is preferentially expressed in high grade tumours. Its expression has no correlation with tumour size and nodal metastasis, which could aid in early treatment and prolonged survival rate among patients independent of other prognostic parameters.

1. Introduction

Cancer is a group of diseases that cause cells in the body to change and spread out of control. Cancer incidence and mortality are rapidly growing worldwide.

Mammary gland is a highly dynamic organ that develops through branching morphogenesis in puberty, evolves during menstrual cycle and undergoes terminal differentiation/dedifferentiation during pregnancy, lactation and involution.

Neoplasm of the breast is the most common malignancy in women around the world. In 2008, 8 million deaths were recorded as a result of malignant diseases, and this figure is estimated to reach 11 million by 2030.[1,2]

Earlier cervical cancer was the most common cancer in Indian women but now the incidence of breast cancer has surpassed cervical cancer and is leading cause of cancer death, although cervical cancer still remains most common in rural India.[3] Changes in risk factors have led to an increase in the prevalence of breast cancer. According to GLOBOCAN 2018 there were 2 million new cases in 2018

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accounting for 25% of all new cancer cases in women.

Breast cancer is a multifactorial disease[1] and various factors contribute to its occurrence. Many studies and experiments demonstrated that carcinomatous cells acquire mesenchymal phenotype and express mesenchymal markers. That is high grade epithelial tumors have shown loss of epithelial morphology and acquire mesenchymal characteristics termed as Epithelial mesenchymal transition (EMT) that correlate with invasiveness and metastatic potential of tumor. Cells proceeding EMT exhibit down-regulation of epithelial markers and cause up regulation of mesenchymal markers such as Vimentin and fibronectin.[4,5]

Vimentin, a class 3 intermediate filament is ubiquitously expressed in normal mesenchymal cells. It is known to maintain cellular integrity and provide resistance against stress. Vimentin is attached to nucleus, endoplasmic reticulum, and mitochondria either laterally or terminally. Vimentin is a multifunctional protein and its ability to interact with large number of proteins, makes it a potential regulator of several different physiological functions. Increased vimentin expression has been reported in various epithelial cancers including prostate. Breast, CNS tumors, GIT tumor, malignant melanoma and lung cancers.[6]

Many literatures have shown that Vimentin expression in breast tumors is one of the important prognostic indicators. Vimentin positive cells are associated with high grade tumors, increased tumor proliferation[6] and also with low PR, low ER, increased basement membrane invasiveness and drug resistance.[7]

So, in our study we analyzed the expression of Vimentin using immunohistochemistry in breast carcinoma. We also studied the expression of this marker in relation to histopathological grade, tumor size and lymph node metastasis.

2. Objectives of the Study

1. To assess the expression of vimentin in various types of breast carcinomas.
2. 2. To find out the correlation between expression of vimentin and prognostic markers such as tumor size, tumor grade and lymph node status.

3. Materials and Methods

Data for the present study was obtained from lumpectomy specimens, received in the department of pathology during the period from December 2017 to May 2019. Patients demographic data such as age, sex, mode of presentation e.g. lump/nipple discharge side, Procedure, tumor size on gross examination, histologic subtype of breast cancer and axillary nodal status. Ethical clearance obtained from ethical committee of our college.

The haematoxylin and eosin (H&E) sections of the cases were taken and reviewed for histological type, histological grade, and lymph node status. Grading was calculated according to Nottingham grading system. All sections were screened for representative tumor paraffin blocks and selected for IHC (Immunohistochemistry).

The representative neoplastic tissue blocks were cut at 4 microns, taken in coated slide & incubated at 58C overnight. Sections were deparaffinized and dehydrated in Xylene, absolute alcohol, 90% and 70% alcohol. Antigen retrieval was done by placing the slides with tri-sodium citrate buffer solutions in microwave and were covered with peroxide block to quench endogenous peroxidase activity. The Sections were stained with primary antibody for one hour. The peroxidase antiperoxidase method was followed for secondary staining and DAB used for colouring antigen-antibody complex.

4. Results

The development of brown colour was interpreted as positive and scoring was done using the method mentioned below.

4.1. Evaluation of immuohistochemical staining

The most representative tumour areas were selected for scoring immunostaining pattern. The scoring was done using light microscopy. Cytoplasmic granular staining was considered as positive reaction for Vimentin. Cut off of positivity in 10% tumor cells was taken as positivity (Table 1).

Table 1: Immunoreactive score for vimentin expression

Score	Vimentin expression
0	Negative with no staining of tumor cells
1+	Weak staining of more than 10% of tumor cells
2+	Moderate staining of more than 10% of tumor cells
3+	Strong staining of more than 10% of tumor cells

Score of 1+ or more was considered as positive

In the present study, more number of cases were in the age group of less than 40 years and least were in age >70 years of age group. The age of the patients ranged from 28 to 90 years. Mean age of cases in our study was 51.0 years. Among vimentin positive tumors age of younger patient was 29 years and that of older was 75 years.

In the study we found no significant distribution of cases related to side of carcinoma and almost all cases of breast carcinoma were diagnosed as invasive carcinoma breast NST and 2 cases of carcinoma with medullary features and mucinous carcinoma, and only 1 case of each invasive papillary carcinoma, lobular carcinoma and mixed type invasive and lobular carcinoma.

Grading was according to WHO 2012 guidelines.(by using Elston's modification of the Bloom and Richardson method). Out of 50 cases 18 cases were Grade 1/

well differentiated carcinoma, 24 cases were grade 2/ moderately differentiated and 8 cases were grade 3/ poorly differentiated carcinoma. Hence majority of cases were moderately differentiated / grade 2 carcinoma.

Invasive tumor size ranged from 2cm to 9cm with mean of 5cm. Maximum cases, 36(72%) out of 50 ranged between tumor size 2-5cm and 13 cases(26%) were of tumor size >5cm with only 1 case showed tumor size <2cm.

Nodal metastasis was found in 40 out of 50 cases with average involvement of four lymph nodes. The number of lymph nodes harvested per case ranged from 0 to 14 were we found 26 cases involving lymph nodes >4, 14 cases shows metastatic deposits in 1-3 lymph nodes, and ten cases shows no deposits.

In our study, vimentin expression was significant in 23/50(46%) cases. Tumors were considered positive when there was distinct brown cytoplasmic staining in cancer cells.

Positive staining in fibroblasts, endothelial cells, lymphocytes and macrophages and negative staining of epithelial cells in non-neoplastic tubules served as 'built in' positive and negative controls respectively.

Table 2: Score wise distribution of vimentin expression in breast carcinoma

S.No.	Vimentin score	No of cases (total 50)	% of cases
1	Score0/Negative	27	54
2	Score 1+	1	2
3	Score 2+	21	42
4	Score 3+	1	2

All 50 cases were taken for immunohistochemistry staining, analysis of vimentin expression and scoring was done to grade intensity of expression. Distinct brown granular cytoplasmic expression was considered as positive for vimentin expression. Out of 50 cases, 27 cases showed negative/ score 0, 1 cases had score 1+ expression, 21 had 2+ expression and 1 had score 3+ expression.(Table 2)

Vimentin is preferentially expressed in invasive carcinoma breast NST(49%) (Figure 1) and carcinoma with medullary features(100%) and not detected in invasive papillary carcinoma (Figure 2), lobular carcinoma, mixed type invasive and lobular carcinoma and mucinous carcinoma (Figure 3).

Grade 3/poorly differentiated tumors showed positivity in 6/8 cases, while 15/24 grade 2/moderately differentiated cases and only 2/18 cases showed vimentin positivity.

Correlation of vimentin expression was done with grade of the tumor. Grade 1 carcinoma showed predominantly score 0 expression. Where as grade 2 and grade 3 carcinoma showed predominantly score 2+ expression. Significant correlation was found between grade of the carcinoma and vimentin expression in breast carcinoma with P value < 0.05.

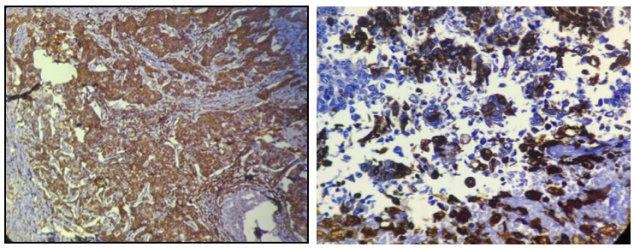

Fig. 1: Poorly differentiated invasive carcinoma breast NST showing vimentin positivity in 60% of tumor cells (DAB, x10)

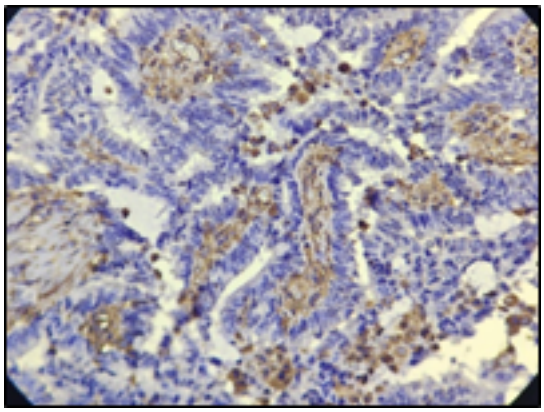

Fig. 2: Invasive papillary carcinoma showing no Immunostaining for vimentin (DAB, x40)

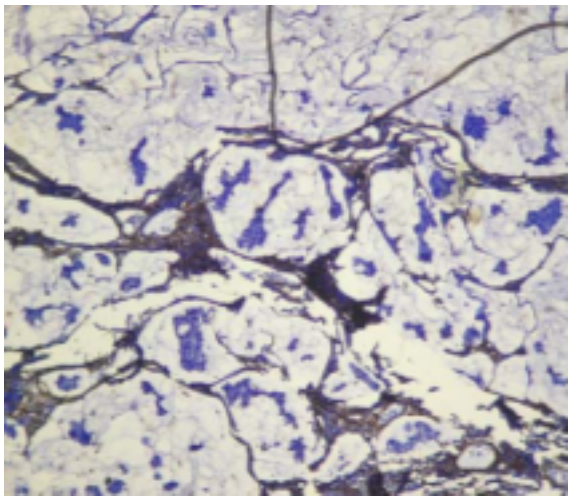

Fig. 3: Mucinous carcinoma showing no Immunostaining for vimentin (DAB, x40)

No significant statistical correlation was found between vimentin expression and size of the carcinoma with P value>0.05.

Correlation of vimentin expression was done with total number of lymph node involved. No significant statistical correlation was found between vimentin expression and lymph node involvement in the present study with P value > 0.05.

5. Discussion

Breast cancer has a diverse clinical spectrum. Despite similarities in the histologic presentation at the time of disease diagnosis, their clinical behaviors, including time to disease progression and metastasis, cannot be predicted with certainty.

Breast cancer is a heterogeneous disease that consists of multiple molecular subtypes. The presence of hormone receptors ER and PR and over expression of human EGFR-2(HER-2) is of central importance in the therapeutic decision making process for patients with breast cancer. Apart from predicting response to therapy, these factors may also determine the likelihood of disease relapse. Hormone positive receptors have been considered to have favorable outcome were as triple negative breast cancers tend to have poor prognosis because of their lack of expression of ER, PR and HER-2 and lack targeted therapies. As a result, the interest in this aggressive TNBC from both clinicians and scientists has grown exponentially.

Therefore, there is dire need to identify the aggressive phenotype such as presence of EMT(epithelial mesenchymal transition) type cells, which would help to predict the behavior of cancer cells. EMT phenomenon is triggered by the interplay of several extracellular signals; many secreted soluble factors, growth factors, their effectors, and many transcription factors including PDGF, Notch and NF KB.[8] The translational relevance of these EMT markers has not been well established, and thus in the present study we interrogated EMT marker vimentin in breast cancer patients. It was Wendy A Raymond et al. in 1989 who first described the expression of vimentin in breast carcinoma.[9]

Age is an important factor in occurrence of carcinoma breast occurring in young. The age of the patients ranged from 28 to 90 years. Mean age of cases in our study was 51.0 years which is slightly more than WHO statistics who have described peak age of 45-50 years in Indian population.[6]The mean age of patient who presented with well differentiated, moderately differentiated and poorly differentiated was 55.2, 49.2and 45.5 respectively.

Vimentin expression was significant in 23/50(46%)cases. Our finding was lesser than Thomas et al[10] who noted positivity in 25/53 cases(47.1%), and found significant association of vimentin expression with high histological grade 3 tumors as well as with ki67 and EGFR expression. Other studies have demonstrated vimentin expression in tumor cells varying from 18%, 22.7%, 17.4% and 18%.

A majority of the above studies considered a cut off of positivity in 10% tumor cells as significant positivity. Even we considered positivity in more than 10% of tumor cells as cutoff point in our study.

5.1. Vimentin and histologic type

Our data suggest that vimentin expression is unevenly distributed among various histological types of breast carcinomas. Overall percentage of vimentin positive breast carcinomas ranged from 49% to 100% in invasive carcinoma breast NST and in carcinoma with medullary features, were as invasive papillary carcinoma, lobular carcinoma and mucinous carcinoma showed no vimentin expression.

Domagala et al.[7] in their study concluded that vimentin expression in breast carcinoma seems to be associated with ductal rather than lobular differentiation. It is preferentially expressed in medullary carcinoma, infiltrative ductal NOS carcinoma of high histologic grade tumors and none of the lobular carcinomas expressed vimentin. Rakshith et al. in their study also found vimentin expression in invasive carcinoma breast NST, mucinous carcinoma but vimentin negativity in lobular carcinoma breast.

Grading of cancer reveals its aggressiveness and is evaluated on the basis of morphology and behavior of cells. Grade 1 and Grade 2 tumors were observed to be predominant in the present study, indicating low grade carcinoma among the patients. Expression of vimentin was significant with grade 3 tumors which was in accordance with study conducted by Hemalath et al,[6] Rakshith et al.,[6] Korsching et al.[10](Table:3). The results were in accordance with the theory of EMT where low-grade cancer cells are known to retain their adhesion properties due to the presence of E-cadherin and alike proteins. With increase in grade the cells undergo transition to mesenchymal cells with loss of adhesiveness, producing higher amount of vimentin.[12]

An increase in vimentin expression is associated with loss of epithelial keratin is an indicator of progressive breast cancer.[13] Additionally Vora et al.[13] in their study opine that in relation to disease status, gain of vimentin was higher in patients who developed recurrence or metastatic disease than in those who did not.

In our present study, invasive tumor size ranged from 3cm to 9cm with mean of 5cm. 1 case with tumor size <2 cm were positive for vimentin, while majority of vimentin positivity was seen in tumor size between 2-5cm. No significant correlation was seen between vimentin expression and tumor size. This negative correlation has also been described by Domagala et al.,[7] Hemalat and Rakshith V et al.[6] The pathological size of a tumor which correlates directly with survival of patients showed no significant difference in vimentin expression.

Table 3: Comparison of vimentin and tumor grade with other studies

S.No.	Study	Grade 1	Grade 2	Grade 3
1	Raymond and Leong [9]	1/16(6.2)	2/23(8.7)	5/11(45.5)
2	Domagala et al [7]	0/4(0)	0/34(0)	15/28(53.5)
3	Korsching et al [10]	-	-	19/21(90.5)
4	Sheshadri et al [11]	4/38(10.5)	10/105(9.5)	26/86(30.2)
5	Hemalatha et al [6]	0/22(0)	2/20(10)	7/8(87.5)
6	Rakshith et al	1/22(4.5)	9/24(37.5)	8/12(66.5)
7	Present study	2/18(11)	15/24(62)	6/8(75)

Axillary lymph node status remains most accepted prognostic factor in breast carcinomas. However, detection of positive lymph nodes may occur late in tumor progression, and negative lymph node may not necessarily exclude aggressive disease or distant metastasis. [14] Of the 23 vimentin positive cases in primary tumor of our study, 17(34%) cases showed metastases in the lymph node and found no statistically significant correlation with nodal metastasis which was concordant with many other studies, expect few studies by Vora et al. [9] in their study showed that vimentin expression was higher in patients with lymph node positive status.

6. Clinical Significance of EMT

Cells that undergo EMT phenomenon may gain metastatic potential but these may account only a small proportion of the total population of tumor cells. Tumor budding is one which involves single cancer cell or small cluster of cells at the invasive front of tumor tissues. Cancer cells in tumor buds have proven for down regulation of E cadherin and increase the expression of vimentin and also possess the characteristics of cancer stem cells. [15,16]

It has been proven that the cells that undergo EMT has developed the ability to invade and acquire resistance for most of the anticancer drugs by various stress conditions such as exposure to radiation and hypoxic conditions. [17,18] Various types of targeted therapy are developed and used against many carcinomas leading to improved survival rate and clinical outcome. [19,20] But however EMT had been reportedly conferring resistance to these targeted agents. Thus it has been getting proven that EMT is leading to resistance to multiple drugs and permitting rapid progression of the tumor. Thus thereby clarifying the correlation between the mechanism of EMT and drug resistance may help clinicians select an optimal anticancer drug treatment and also choose the mode for high risk cases of invasion.

7. Conclusion

Taken together, the concept of EMT is a valuable guide for the morphologic and molecular changes observed in tumor cell invasion and metastasis. Association between EMT like cellular phenotype as shown by changes in marker protein expression and tumor aggressivity has well proven in malignancy. Accordingly, in our study, high grade tumors preferentially expressed vimentin and showed no correlation with tumor size and nodal metastasis.

For future significance of vimentin as biomarker for malignancy with clinical relevance, more research will be necessary to assess the major functions of vimentin in tumorigenesis. In view of available data, vimentin expression in cancer is likely to become an attractive and promising therapeutic target and has great potential for providing novel clinical prognostic and diagnostic tools. Further, the use of vimentin specific chemical inhibitors as well as novel therapeutic agents directed against vimentin in combination with other anticancer agents, must be encouraged.

8. Source of Funding

None.

9. Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Momenimovahed Z, Salehiniya H. Epidemiology characteristics of and risk factors for breast cancer in the world. *Breast Cancer*. 2019;1:151–64.
2. Benson JR, Jatoi I. The global breast cancer burden. *Future Oncol*. 2012;8(6):697–702. doi:10.2217/fon.12.61.
3. Malvia S, Bagadi SA, Dubey US, Saxena S. Epidemiology of breast cancer in Indian women. *Asia-Pac J Clin Oncol*. 2017;13(4):289–95. doi:10.1111/ajco.12661.
4. Huber MA, Kraut N, Beug H. Molecular requirements for epithelial–mesenchymal transition during tumor progression. *Curr Opin Cell Biol*. 2005;17(5):548–58. doi:10.1016/j.ceb.2005.08.001.
5. Christiansen JJ, Rajasekaran AK. Reassessing Epithelial to Mesenchymal Transition as a Prerequisite for Carcinoma Invasion and Metastasis. *Cancer Res*. 2006;66(17):8319–26. doi:10.1158/0008-5472.can-06-0410.
6. Hemalatha A, Suresh TN, Kumar MLH. Expression of vimentin in breast carcinoma, its correlation with Ki67 and other histopathological parameters. *Indian J Cancer*. 2013;50(3):189–94. doi:10.4103/0019-509x.118724.
7. Domagala W, Losata J, Bartkowiak J, Weber K, Osborn M. Vimentin is preferentially expressed in human breast carcinomas with low estrogen receptor and high ki67 growth fraction. *Am J Pathol*. 1990;136:219–27.

8. Wang Z, Li Y, Kong D, Banerjee S. Acquisition of epithelial-mesenchymal transition phenotype of gemcitabine-resistant pancreatic cancer cells is linked with activation of the notch signaling pathway. *Cancer Res*. 2009;69:2400–7.
9. Raymond WA, Leong AS. Vimentin - A new prognostic parameter in breast cancer? *J Pathol*. 1989;158:107–14.
10. Korsching E, Packeisen J, Liedtke C, Hungermann D, Wülfing P, Diest PJ, et al. The origin of vimentin expression in invasive breast cancer: epithelial–mesenchymal transition, myoepithelial histogenesis or histogenesis from progenitor cells with bilinear differentiation potential? *J Pathol*. 2005;206(4):451–7. doi:10.1002/path.1797.
11. Seshadri R, Raymond WA, Leong ASY, Horsfall DJ, McCaul K. Vimentin expression is not associated with poor prognosis in breast cancer. *Int J Cancer*. 1996;67(3):353–6. doi:10.1002/(sici)1097-0215(19960729)67:3<353::aid-ijc8>3.0.co;2-q.
12. Crook ET, Thompson EW, Thiery JP. Epithelial to mesenchymal transition and breast cancer. *Breast Cancer Res*. 2009;11(6):213. doi:10.1186/bcr2416.
13. Vora HH, Patel NA, Rajvik KN, Mehta SV, Brahmbhatt BV, Shah MJ. Cytokeratin and vimentin expression in breast cancer. *Int J Biol Mark*. 2009;24(1):38–46.
14. Thomas PA, Kirschman DA, Cerhan JR, Folberg R, Seftor EA, Sellers TA. Association between keratin and vimentin expression, Malignant phenotype, and survival in postmenopausal breast cancer patients. *Clin Cancer Res*. 1999;5:2698–703.
15. Garber K. Epithelial-to-Mesenchymal Transition Is Important to Metastasis, But Questions Remain. *J Natl Cancer Inst*. 2008;100(4):232–9. doi:10.1093/jnci/djn032.
16. Masaki T, Goto A, Sugiyama M, Matsuoka H, Abe N, Sakamoto A, et al. Possible contribution of CD44 variant 6 and nuclear ?-catenin expression to the formation of budding tumor cells in patients with T1 colorectal carcinoma. *Cancer*. 2001;92(10):2539–46. doi:10.1002/1097-0142(20011115)92:10<2539::aid-cncr1605>3.0.co;2-i.
17. Wahid A, Ahamd S, Sajjad M. Pattern of carcinoma of oral cavity reporting at dental department of Ayub medical college. *J Ayub Med Coll Abbottabad*. 2005;(1):65–6.
18. Jung JW, Hwang SY, Hwang JS, Oh ES, Park S, Han IO. Ionising radiation induces changes associated with epithelial-mesenchymal trans differentiation and increased cell motility of A549 lung epithelial cells. *Eur J Cancer*. 2007;43:1214–24.
19. Shah AN, Summy JM, Zhang J, Park SI, Parikh NU, Gallick GE. Development and Characterization of Gemcitabine-Resistant Pancreatic Tumor Cells. *Ann Surg Oncol*. 2007;14(12):3629–37. doi:10.1245/s10434-007-9583-5.
20. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, et al. Bevacizumab plus Irinotecan, Fluorouracil, and Leucovorin for Metastatic Colorectal Cancer. *N Engl J Med*. 2004;350(23):2335–42. doi:10.1056/nejmoa032691.

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