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Original Article

Expression of vimentin in breast carcinoma, its correlation with Ki67 and other histopathological parameters

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Abstract

CONTEXT: Vimentin is a mesenchymal marker, known to express in some epithelial carcinomas. **AIMS:** 1. To find out the expression of vimentin in infiltrating ductal carcinoma of breast (not otherwise specified), 2. To find out the correlation between expression of vimentin and prognostic markers such as tumor size, tumor grade, lymph node status, proliferation index (measured by Ki 67), and Nottingham prognostic index (NPI). **MATERIALS AND METHODS:** Study was done at Department of Pathology; 50 cases of infiltrating ductal carcinoma (NOS) were studied for tumor grade; immunohistochemistry was done using antibodies against vimentin and Ki 67. Percentages of positive cells were documented. An immunoscore was also calculated for vimentin. Vimentin expression was correlated with tumor size, lymph node status, Nottingham prognostic index, and Ki 67. Statistical analysis used: statistical correlation was done using Pearson's chi-square test. A *P* value less than 0.01 was considered significant. **RESULTS:** Vimentin expression was seen in 18% of cases. Its expression correlated with high tumor grade and high growth fraction (*P* value < 0.01). It did not correlate with lymph node status, tumor size, and NPI. **CONCLUSIONS:** Increased vimentin expression is associated with bad prognostic factors. Immunohistochemistry with vimentin may be helpful in knowing the prognosis in cases of infiltrating ductal carcinoma of breast (NOS).

Key words: Breast carcinoma, grade, Ki 67, lymph node status, tumor size, vimentin

Introduction

Carcinoma of breast is one of the leading cancers occurring in women in India. Breast carcinoma is the second most common cancer (after cervical cancer) with an estimated 115,251 new diagnoses and the second most common cause of cancer-related deaths with 53,592 breast cancer deaths in 2008.[1] Conventional prognostic factors such as tumor size, histological type, differentiation, microscopic grade, lymph node status, tumor necrosis, hormone receptor status, newer markers such as Her-2 neu, proliferative indices, p 53, markers of angiogenesis such as factor VIII, CD31, and CD 34, invasion markers such as cathepsin D, urokinase plasminogen activator (uPA) have been described in assessing the prognostic and therapeutic outcome in these patients.[2]

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Vimentin is an intermediate filament expressed in tissues of normal mesenchymal origin. It is known to express aberrantly in epithelial cancers of prostate, gastrointestinal tract, breast, central nervous system, lung, and malignant melanomas.[3] In breast carcinomas, it is known to be expressed significantly in high grade infiltrating ductal carcinoma, medullary carcinomas but not in lobular carcinomas of breast.[4] In infiltrating ductal carcinoma, expression of vimentin is associated with low ER, low PR, increased basement membrane invasiveness, and drug resistance.[5-7]

Total proliferative activity of tumor is one of the important prognostic markers which decide the neoadjuvant therapy in patients with breast carcinoma. It is measured by counting mitotic figures on hematoxylin and eosin-stained section, sections stained

with silver stains like AgNOR, monoclonal antibodies against Ki 67, thymidine labeling index, and DNA flow cytometry. Among all the available methods, immunohistochemistry with monoclonal antibodies against Ki 67 has been increasingly used, as it is simple, reliable, and helps in rapid assessment of total proliferative activity.[8]

This study was taken up with an aim of (1) to assess the expression of vimentin in infiltrating ductal carcinoma of breast (NOS – not otherwise specified), and (2) to study the correlation between expression of vimentin and prognostic markers such as tumor size, tumor grade, lymph node status, proliferation index (measured by Ki 67), and Nottingham prognostic index (NPI).

Materials and Methods

This is a retrospective study carried out in the Department of Pathology, of our institute. Fifty cases of infiltrating ductal carcinoma (NOS) were taken up for this study. Patient's demographic data such as age, details of tumor size, lymph node status, paraffin blocks, and slides were retrieved from archives of our department. All cases of male breast carcinoma and carcinomas of other histological types were excluded from this study.

Ethical clearance was obtained from the ethical committee of our college. All cases were reviewed for histological type, histological grade, and lymph node status. Grading was calculated according to Nottingham grading system.

Immunohistochemistry (IHC)—All sections were screened and representative blocks were selected for IHC. Three to four micrometer thin sections were taken on slides coated with 1% organosilane. Sections were deparrafinized and dehydrated in xylene, absolute alcohol, 90% and 70% alcohol. Antigen retrieval was done in microwave using tri sodium citrate buffer at pH 6. Sections were treated with 3% hydrogen peroxidase to quench endogenous peroxidase activity. Sections were stained with antibodies against Ki 67 for 2 h and antibodies against Vimentin for 45 min, (positive and negative controls were run simultaneously). The peroxidase antiperoxidase method was followed for secondary staining. DAB was used for coloring the antigen–antibody complex. (Primary and secondary antibodies were obtained from Biogenix USA).

IHC slides were initially screened under 40 magnifications to identify areas of maximum intensity.

Ki 67 scoring—Sections were examined under 400 magnification. Distinct nuclear staining was taken as positive. (Germinal centre of reactive lymph node was taken as positive control). (Image 1 shows Ki 67 nuclear positivity in 40% of tumor cells IHC 100x. Inset shows distinct nuclear staining IHC 400x), Image 3 shows Ki 67 nuclear positivity in 60% of tumor cells IHC 100x. Inset shows distinct nuclear staining IHC 400x). Five hundred cells were counted in an area of maximum Ki 67 positivity and expressed as percentage. A count of more than 15% was taken as significant.

Vimentin scoring—Sections were examined under 400 magnification. Distinct granular cytoplasmic staining was taken as positive (fibroblasts, endothelial lining were taken as positive control). (Image 2 shows vimentin cytoplasmic positivity in 60% of tumor cells IHC 100x. Inset shows distinct cytoplasmic positivity IHC 400x). Five hundred cells were counted in an area of maximum vimentin positivity. A score of more than 10% was considered as significant.

Immunoscore was calculated for vimentin-stained slides according to formula:
Immunoscore = % of positive cells x staining intensity [no staining (0), weak (1+), moderate (2+), strong (3+)]. A score of more than 30 was considered significant.

The expression of both Ki67 and vimentin was evaluated by two observers independently and they were blinded to the clinical and histopathological data at this stage. A consensus was sought for differences of opinion from third author.

Size of the tumor was classified as T1, T2, and T3 and lymph node status was classified as pN1, pN2, and pN3 according to the TNM staging system.

NPI was calculated in 37 cases as follows.

Tumor size in cm x 0.2 + lymph-node stage (I, II or III) + histologic grade (1, 2 or 3) and categorized as follows: A < 3.4 – Good prognosis, B > 3.4 and < 5.4 – Moderate prognosis, C > 5.4 – Poor prognosis group. Statistical analysis was done by using SPSS 15. Statistical correlation was calculated using Pearson's chi-square test. A P value < 0.01 was considered significant

Results

Most of the patients were in the age group of 45 to 54 years (38%). Youngest patient in our series was 35

years, while the oldest was 84 years. The mean age was 51.2 years. Out of 50 cases, 22 cases (44%) belonged to grade 1, 20 cases (40%) were of grade 2, and 8 cases (16%) were of grade 3.

Vimentin expression was positive in 9/50 (18%) cases. Among the vimentin-positive tumors age of youngest patient was 35 years and that of oldest was 65 years with a mean age of 45.6 years. Eight out of twenty-seven patients (8/27) aged less than 50 years showed vimentin positivity, while one out of twenty-three (1/23) patients were aged more than 50 years and showed vimentin positivity. Grade III tumors showed positivity in 7/8 tumors, while only 2/20 grade II tumors, and none of the grade 1 tumors were vimentin positive. Ki 67 was significantly positive in 28/50 (56%) of tumors. All nine tumors with vimentin positivity showed significant Ki 67 positivity.

Tumor size ranged from 1 to 10 cm with a mean size of 5 cm. Status of nodal metastasis was available in 37 cases. No deposit were seen in 7 cases , deposits in less than 3 nodes were seen in 17 nodes, deposits in less than 9 nodes were seen in 12 cases, deposits in 10 nodes were seen in 1 case. The expression of vimentin and other and prognostic marker expression is represented in Table 1.

Age, tumor size, nodal status, grade of tumor, Ki 67 %, vimentin %, vimentin immunoscore of nine significant cases are mentioned in Table 2.

Significant correlation was present between vimentin expression with tumor grade and Ki 67 ($P < 0.01$), while no significant correlation was found between vimentin expression with tumor size, lymph node status, and NPI.

Discussion

Traditionally vimentin has been used as a mesenchymal marker. It is also used in immunohistochemistry to assess the extent of antigen damage occurring in tissues due to fixation and processing.[9] It was Wendy A Raymond *et al.* in 1989 who first described the expression of vimentin in breast carcinomas.[4]

Age is an important factor in occurrence of carcinoma breast with carcinoma rarely occurring in young. Mean age of cases in our study was 51.2 years which is slightly more than WHO statistics who have described peak age of 45–50 years in Indian population.[10]

In our study, vimentin expression was significant in 9/50 (18%) cases. Vimentin expression in tumor cells has been reported to be 7.7%, 21.1%, 14%, and 13.4% by various authors.[5,11-13]. Our finding was lesser than Thomas *et al.* who noted positivity in 25/53 (47.1%) cases.[14] This may be because they have considered all subtypes of carcinoma breast and not just IDC (NOS). A majority of the studies concerning vimentin expression have considered a cut off of positivity in 10% tumor cells as significant positivity.[5,15,16] Even we considered positivity in more than 10% of tumor cells as a cutoff point in our study. Niveditha *et al.* have opine than a cutoff value of 10 will help us in segregating non tumorous cells like stromal cells that are caught within the tumor nest.[5] Eight of the patients with significant vimentin positivity were seen in females in their golden years (50 years) and one case in a 65 year old lady. An overall mean young age (45.6 years) has also been documented by Kusinska *et al.* and Chen *et al.*[11,17]

Tumor grade is an important prognostic factor in carcinoma breast. The comparison of our findings with other studies is in Table 3.

Ki 67 antigen was originally identified by Gerdes and his colleagues in early 1980s. American society of clinical oncology does not include Ki 67 assessment as a part of their existing guidelines as routine biological marker that can be used in treatment of breast carcinoma. However, its role as a prognostic marker for breast carcinoma is undisputed as it serves as a

Table 1: Expression of vimentin and other prognostic marker expression

	Vimentin		
	n (50)	Significant positive (>10% of Cells)	*P* value
Tumor grade (Nottingham grading)			
I	22	0	
II	20	2	0.000*
III	08	7	
Ki 67 (MIB-1)			
<15%	22	0	0.000*
>15%	28	9	
Tumor size			
T1	8	1	
T2	22	4	0.670
T3	20	4	
Lymph node (n = 37)			
NO	7	2	
N1	17	3	0.482
N2	12	2	
N3	1	0	
NPI (n = 37)			
A	9	2	
B	20	4	0.627
C	8	1	

Table 2: Age, tumor size, nodal status, grade of tumor Ki 67 levels, vimentin and vimentin immunoscore

Sl no	Age	Maximum tumor size	Lymph node status	Grade	Ki 67 %	NPI	Vimentin %	Vimentin immunoscore
1	35	3	0/4	III	50	4.6	40	80
2	50	3.5	4/11	III	50	6.7	20	40
3	47	9	2/14	III	60	6.8	20	40
4	50	7	NA	III	40	NA	60	180
5	40	9	2/2	II	30	5.8	15	45
6	41	2.5	NA	III	40	NA	20	40
7	46	8	4/9	II	50	7.6	15	45
8	36	3	1/4	III	60	4.6	20	40
9	65	2	2/2	III	50	5.4	15	30

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

	Grade I (%)	Grade II (%)	Grade III (%)
Raymond and Leong[4]	1/16 (6.2)	2/23 (8.7)	5/11 (45.5)
Domagala *et al.*[16]	0/4 (0)	0/34 (0)	15/28 (53.5)
Korsching *et al.*[13]	-	-	19/21 (90.5)
Sheshadri *et al.*[12]	4/38 (10.5)	10/105 (9.5)	26/86 (30.2)
Our Study	0/22 (0)	2/20 (10)	7/8 (87.5)

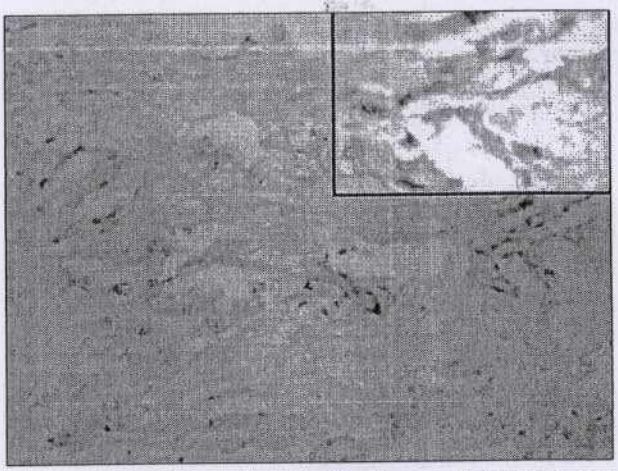

Figure 1: Ki 67 nuclear positivity in 40% of tumor cells IHC 100x. Inset shows distinct nuclear staining IHC 400x

predictive tool in identifying patients who can benefit from chemotherapy or endocrine treatment.[18] A count of 15% of Ki 67 was considered as cut off in our study because a recent study have observed that Ki 67 values above 10–14% defines a high-risk group in breast carcinoma patients making these patients successful candidates for neoadjuvant therapy.[18] In our study, vimentin expression was significantly associated with high growth fraction. In 6/9 vimentin positive tumors Ki 67 was positive in more than 50% of tumor cells. Our findings correlated with other studies by Raymond *et al.*, Domagala *et al.*, and Thomas *et al.*[4,14,15]

No significant correlation was seen between tumor size and lymph node status. This negative correlation has also been described by Domangala *et al.* and Niveditha *et al.*[4,15] 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 metastases.[14] Hence, a new marker that can predict the aggressive phenotype is important.

Nottingham prognostic index is a well-established widely used method of predicting survival of operable primary breast carcinoma cases.[19] We did not find any significant correlation between vimentin expression and NPI. Even Sheshadri *et al.* found a negative correlation between vimentin expression and survival status.[12] Thomas *et al.* have opined that co expression of vimentin and keratin is more important in predicting the survival rather than expression of either of the intermediate filaments alone.[14]

Most of the studies have considered the percentage of vimentin-positive cells without much emphasis on the intensity of staining (as followed in ER, PR). Hence, we wanted to assess the role of intensity of staining in prognosis of disease by calculating immunoscore. However, statistical analysis of vimentin immunoscore versus the prognostic parameters used in this study did not significantly differ from use of vimentin values alone.

Numerous theories have been put forward to define the role of vimentin in pathogenesis of breast carcinomas.

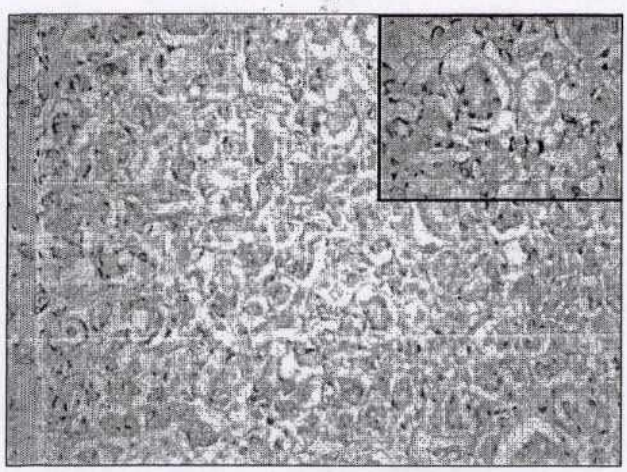

Figure 2: Vimentin cytoplasmic positivity in 60% of tumor cells IHC 100x. Inset shows distinct cytoplasmic positivity IHC 400x

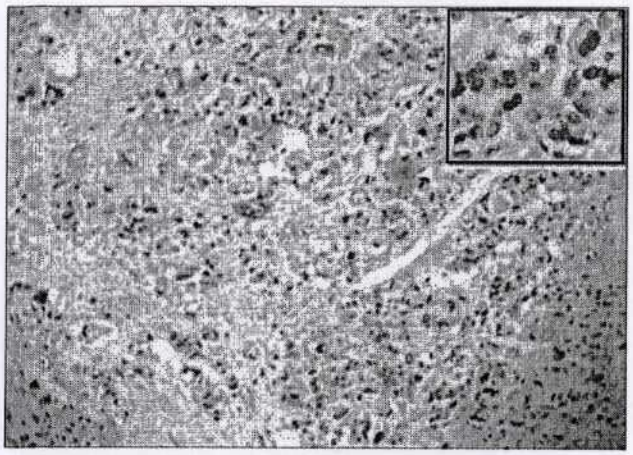

Figure 3: Ki 67 nuclear positivity in 60% of tumor cells IHC 100x. Inset shows distinct nuclear staining IHC 400x)

Initially, epithelial mesenchymal transition of tumor cells was thought to be the mechanism of vimentin expression.[20] Koersching *et al.* have proposed an alternative hypothesis that vimentin expressing breast carcinoma cells are derived from breast progenitor cells with bilinear (glandular and myoepithelial) differentiation potential and not because of epithelial mesenchymal transition.[13]

By virtue of its overexpression in many epithelial carcinomas vimentin expression may serve as an potential target for cancer therapy. Further, with the discovery of drug WFA (Withaferin A) a potent breast anticancer agent that acts by inducing perinuclear vimentin accumulation followed by rapid vimentin depolymerization, concomitant vimentin ser56 phosphorylation at low doses, identifying vimentin-positive cells may have a positive impact in prolonging the life of patients with infiltrating ductal carcinoma (NOS).[21]

Conclusion

Increased vimentin expression is seen in breast carcinomas occurring in females in golden years of age (<50 years). Vimentin-positive cells are associated with high grade tumors, and increased tumor proliferation. Its expression has no correlation with tumor size, nodal metastasis, and survival status (calculated by NPI). Studies involving large population, with long-term follow up are necessary to further define the role of vimentin in patient survival and disease free period. Also, more studies have to be undertaken in defining the exact cut off value of vimentin positivity, role of intensity of staining (immunoscore) in cases of infiltrating ductal carcinoma (NOS).

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