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Original Article

Pathology Section

Assessment of Epithelial to Mesenchymal Transition Markers in Breast Carcinoma and its Association with Histopathological Grading, Size and Metastasis

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ABSTRACT

Introduction: Carcinoma breast is the most common malignancy affecting women worldwide. Early detection is key to its effective management. Epithelial to Mesenchymal Transition (EMT) has implications in progression of breast carcinoma and metastasis.

Aim: To assess use of EMT Immunohistochemical (IHC) markers in breast carcinoma and its association with histopathological grading, size and metastasis.

Materials and Methods: This is a hospital-based descriptive study was carried on patients of breast carcinoma attending the Outpatient Department of Surgery, Jawaharlal Nehru Medical College, AMU, Aligarh, Uttar Pradesh, India, from July 2011 to October 2013. Total of 167 cases of carcinoma breast specimens were received in the department. Histopathological examination of 67 mastectomy specimen was done and graded using Scarff-Bloom-Richardson (SBR) grading system. Lymph nodes found were screened for metastasis. Subsequently IHC study with cytokeratin 5/6 and vimentin were done to assess EMT in 67 out of 167 cases. Statistical analysis was done using Chi-square or Fisher's exact test on Statistical Package for Social Sciences (SPSS) software version 15.0 USA.

Results: Using SBR grading system, 11 (16.4%) cases were of grade I, 34 (50.7%) cases of grade II and 22 (32.8%) cases were of grade III. Out of total, 3/11 (27.3%) of grade I, 23/34 (67.6%) of grade II and 20/22 (90.9%) of grade III tumour were found to have metastasis. Positivity for cytokeratin 5/6 and vimentin were 3/11 (27.3%) and 7/11 (63.6%) respectively in grade I tumours. Grade II tumours showed 2/34 (5.9%) positivity for cytokeratin 5/6 and 21/22 (95.5%) for vimentin. Positivity for cytokeratin 5/6 was 0 and 95.5% (21/22) for vimentin in grade III tumours.

Conclusion: Increase in metastasis was seen with progression of grade. Down regulation of cytokeratin 5/6 and upregulation of vimentin was observed as the grade of tumour increased. Cytokeratin 5/6 and vimentin may be used to assess EMT which in turn shows higher chances of metastasis.

Keywords: Bloom-Richardson grading system, Carcinoma breast, Cytokeratin 5/6, Vimentin

INTRODUCTION

Breast cancer is the most common malignancy affecting women, accounts for 11.7% of the total cancer incidence burden in the world with 2.26 million new cases and about 684,996 deaths in 2020 [1]. Carcinoma of the breast and cervix account for majority of total cases of cancers in Indian women, of which breast carcinoma accounts for 13.5% (178361) of all cancers and 10.6% (90408) of all deaths. Incidence of carcinoma cervix is found to be on decreasing trend and carcinoma breast on the rise [2].

The approach to the management of breast carcinoma has undergone enormous changes over the last few decades. Today, the choice of conservative and reconstructive surgery is more popular than mastectomy. Such changes are accompanied by increasing range of systemic, hormonal and cytotoxic drugs used in both adjuvant and neoadjuvant settings (Adrienne, 2019) [3]. Prognosis and management of breast carcinoma are influenced by the classic variables such as histological type and grade, tumour size, lymph node status, estrogen receptor status and Epidermal Growth Factor Receptor 2 (EGFR2) over expression [4].

The staging systems currently in use for breast cancer are based on the clinical size and extent of invasion of the primary tumour (T), absence or presence of lymph node metastasis and evidence of their local invasion (N), together with the clinical and imaging evidence of distant metastases (M). This is then translated into the TNM classification [5].

Almost all the breast malignancies arise from the epithelial lining of the breast lobes and lobules, which invades the local tissues and undergo distant metastasis. EMT is considered to be an essential process in the metastatic cascade. EMT is a process whereby epithelial cells lose their epithelial characteristics and acquire a mesenchymal phenotype. Invasive and metastatic potential of transformed cells is increased. Down regulation of epithelial markers such as cytokeratin and E-cadherin and upregulation of mesenchymal marker such as vimentin, N-cadherin and cadherin-11 characterises the EMT process [6].

This study investigated the use of cytokeratin 5/6 (epithelial marker) and vimentin (mesenchymal marker) in cases of carcinoma breast and their role in EMT process and their assosciation with respect to tumour size, tumour grade and metastasis.

MATERIALS AND METHODS

This hospital-based descriptive study was carried on patients of breast carcinoma attending the Outpatient Department (OPD) of Surgery, Jawaharlal Nehru Medical College, AMU, Aligarh, Uttar Pradesh, India, July 2011 to October 2013. Ethical clearance was obtained (D1247/GH dated 20/11/2013) from the Institute's Ethical Committee and informed consent from patients was taken.

Inclusion criteria: Any patient who attended surgery OPD during the study period with breast lump(s) or any patient who had a positive family history or comes for screening with or without any palpable breast lump and later diagnosed to have carcinoma breast were included in this study.

Exclusion criteria: Patients who were unwilling to participate in the study were excluded.

Study Procedure

Total of 167 cases of carcinoma breast specimens were received in the department. Detailed history of the patients was taken and proper clinical examination was done. Mastectomy and lumpectomy specimens with or without axillary lymphadenectomy underwent histopathological examination and grading of cases of breast carcinoma was done as per the modified Scarff-Bloom-Richardson grading system [7]. Cases of stromal sarcoma were graded on the basis of FNCLCC (Fédération Nationale des Centres de Lutte Contre le Cancer) grading system [8]. Immunohistochemical (IHC) procedure for cytokeratin 5/6 and vimentin was done by manual technique for 67 out of 167 cases which came during October 2012 to September 2013. Total of 67 cases only were chosen because of financial constrain and was selected by lottery method according to simple random technique.

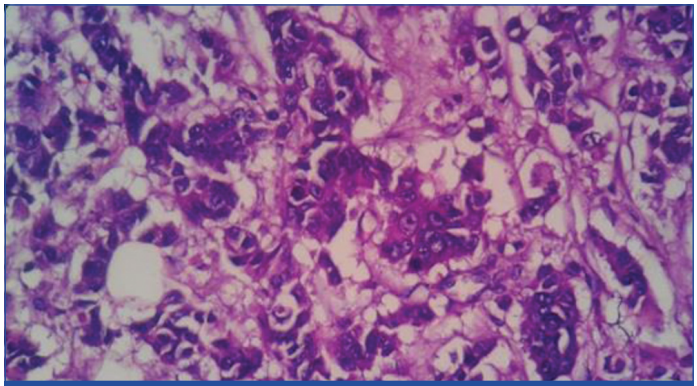
- For cytokeratin 5/6, mouse monoclonal antibody (Thermoscientific, Catalog #MS-1814-S0) was used at 1:10 dilution and squamous cell carcinoma case was used as positive control.
- For vimentin, mouse monoclonal antibody (Thermoscientific, Catalogue #MS-129-P0) was used at 1:50 dilution and used fibroblast as positive control. Both the immunostains were reviewed by two pathologists and were considered positive when more than 10% cells were positive and even if weakly staining in both.

STATISTICAL ANALYSIS

Statistical analysis was done using Chi-square or Fisher's exact test on Statistical Package for Social Sciences (SPSS) software (v.15.0, USA) to evaluate the significance of difference between association of variables like cytokeratin 5/6 and vimentin expression, nodal metastasis, tumour size and tumour grade. A p-value <0.05 was considered statistically significant.

RESULTS

Out of total 67, most of the tumours with IHC were in grade II 34 (50.7%), whereas 22 (32.8%) were in grade III [Table/Fig-1] and 11 (16.4%) in grade I. They showed an increasing trend of metastasis with increase in grade. Nodal metastasis was found in 3/11 (27.3%) of grade I, 23/34 (67.6%) of grade II and 20/22 (90.9%) of grade III tumours [Table/Fig-2].

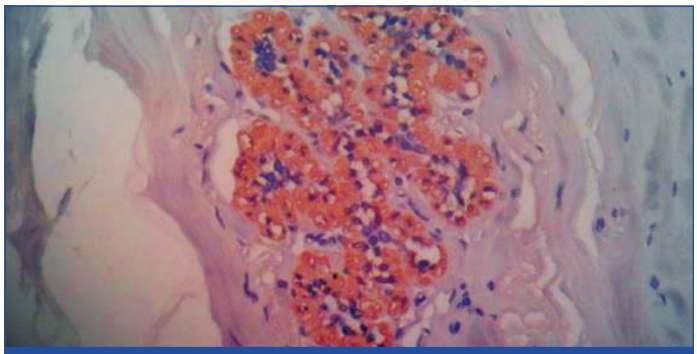


[Table/Fig-1]: Grade III-infiltrating ductal carcinoma (H&E 40X).

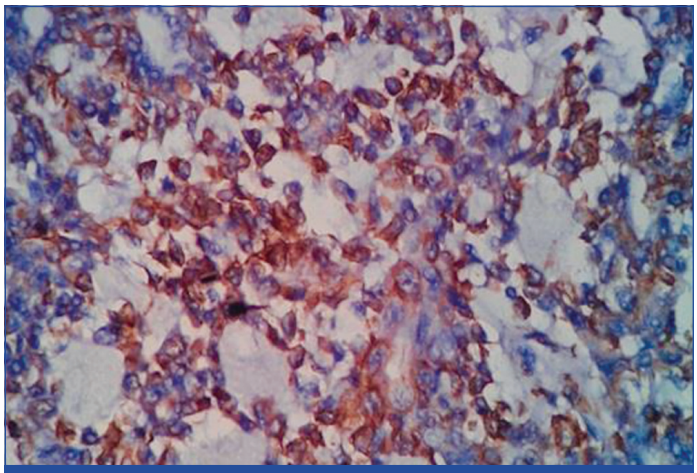
SBR grading	Metastasis		Total cases, n (%)
	Positive, n (%)	Negative, n (%)	
Grade I	3 (27.3%)	8 (72.7%)	11 (16.42%)
Grade II	23 (67.6%)	11 (32.4%)	34 (50.75%)
Grade III	20 (90.9%)	2 (9.1%)	22 (32.83%)
Total	46 (68.7%)	21 (31.3%)	67 (100%)

[Table/Fig-2]: Association between SBR histological grade and lymph node metastasis in cases where immunostaining were performed.
A p-value <0.05 was considered statistically significant; χ^2 value=13.83, df=2, p-value=0.0001. (Significant), (N=67)

There was down regulation of cytokeratin 5/6 [Table/Fig-3] with increase in grade of the tumour (p-value=0.02) and increase in expression of vimentin [Table/Fig-4] with increase of tumour grade (p-value=0.02). Both associations were statistically significant [Table/Fig-5].



[Table/Fig-3]: CK 5/6 positivity in grade I infiltrating ductal carcinoma (H&E 40X).



[Table/Fig-4]: Vimentin positivity in grade III infiltrating ductal carcinoma (H&E 40X).

SBR grading	Cytokeratin 5/6 (Epithelial marker)		Vimentin (Mesenchymal marker)		Total cases n (%)
	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)	
Grade I	3 (27.3%)	8 (72.7%)	7 (63.6%)	4 (36.4%)	11 (16.4%)
Grade II	2 (5.9%)	32 (94.1%)	28 (82.4%)	6 (17.6%)	34 (50.7%)
Grade III	-	22 (100%)	21 (95.5%)	1 (4.5%)	22 (32.8%)
Total	5 (7.5%)	62 (92.5%)	56 (83.6%)	11 (16.4%)	67

[Table/Fig-5]: Association between histopathological grade and expression of cytokeratin 5/6 and vimentin.
A p-value <0.05 was considered statistically significant; Cytokeratin 5/6 value=8.15, df=2, p-value=0.02 (Significant); Vimentin value=7.87, df=2, p-value=0.02 (significant)

Only 1/5 (20%) cases of cytokeratin 5/6 positivity had metastasis whereas 45/62 (72.5%) of cytokeratin 5/6 negative cases had metastasis. Inverse association of cytokeratin 5/6 expression and lymph node metastasis is seen which shows some association (p-value=0.05). Vimentin positive 42/56 (75%) and vimentin negative staining cases 4/11 (36.4%) had lymph node metastasis. Expression of vimentin shows higher number of cases with metastasis which was statistically significant (p-value=0.003) [Table/Fig-6].

Stains		Metastasis		Total	Statistics value
		Positive n (%)	Negative n (%)		
Cytokeratin 5/6	Positive	1 (20%)	4 (80%)	5	p=0.05, χ^2=3.75, df=1
	Negative	45 (72.5%)	17 (27.4%)	62	
Vimentin	Positive	42 (75%)	14 (25%)	56	p=0.03, χ^2=4.71, df=2
	Negative	4 (36.4%)	7 (63.6%)	11	

[Table/Fig-6]: Association between lymph node metastasis and expression of cytokeratin 5/6 and vimentin.

Among 67, 5/17 (29.4%) of T1 tumours showed positivity for cytokeratin 5/6. None of the T2 and T3 tumours were positive for cytokeratin 5/6. This association was statistically significant (p-value=0.0004). A steady increase in expression of vimentin is seen with increase in tumour size. Out of all, 11/17 (64.7%) expression of vimentin were seen in tumours with T1 stage whereas T2 tumour showed 30/35 (85.7%) and T3 tumours showed 15/15 (100%) expression of vimentin. This association was statistically significant (p-value=0.02) [Table/Fig-7].

Tumour size	No. of cases n (%)	CK 5/6 positive n (%)	CK 5/6 negative n (%)	Vimentin positive n (%)	Vimentin negative n (%)
T1: <2 cm	17 (25.4%)	4 (23.5%)	13 (76.5%)	9 (52.9%)	8 (47.1%)
T2: >2 cm but <5 cm	35 (52.2%)	-	35 (100%)	30 (85.7%)	5 (14.3%)
T3: >5 cm	15 (22.4%)	-	15 (100%)	15 (100%)	-
Total	67 (100%)	4 (6%)	63 (94%)	54 (80.6%)	13 (19.4%)

[Table/Fig-7]: Association between cytokeratin 5/6 expression and vimentin expressions with tumour size in cases of breast carcinoma.
χ^2=12.512;df=2; p=0.002; χ^2=12.512; df=2; p=0.002

DISCUSSION

Breast cancer is the most common cancer affecting women worldwide and common cause of cancer related deaths [1]. It has overtaken cancer cervix as the most common cancer among women in some areas of India and is showing an increasing trend. Each year 9,00,000 people are diagnosed with breast cancer worldwide and causes 5,19,000 deaths [9].

Cytokeratin (CK) 5/6 a member of the intermediate filament family of proteins is expressed by the basal/myoepithelial cells. CK 5/6 was used as one of panel of markers in many studies, which also had done on the lesions other than that of breast. Ordonez NG, Chu PG and Weiss LM and Cury PM et al., reported 12.5%, 30.8% and 9.5% positivity of CK5/6 respectively in their studies of breast malignancies [10-12]. Bhalla A et al., had correlated expression of CK 5/6 with various features of breast malignancies like tumour grade, tumour size and lymphatic metastasis etc. in detail [13]. They reported a decrease in expression of cytokeratin 5/6 among malignant lesion with 95% positivity in benign cases as compared to 24% positivity among malignant cases. However, they found expression of cytokeratin 5/6 among malignant cases associated with higher grade of tumour. Present study showed a much lower number of cases (7.5%) to be positive for cytokeratin 5/6.

According to the present study, all CK 5/6 positive cases were ≤2 cm and were of either grade I or II [Table/Fig-4]. But Bhalla A et al., found all malignant cytokeratin 5/6 immunoreactive cases were of 3 cm or more in size [13]. This difference in the study could be because of sensitivity of the antibody used and size of the study group.

Sutton LM et al., found intratumoural expression of CK5/6 was significantly higher in the axillary node positive group (59.06%±9.26%) compared to the axillary node negative group (20.79%±7.45%) [14]. Result of the present study was contrary to those of Sutton LM et al., [14]. The present study showed only 20% cases of cytokeratin 5/6 positivity having lymph node metastasis whereas 72.6% of cytokeratin 5/6 negative cases showed positive lymph node metastasis. An inverse correlation of cytokeratin 5/6 expression and lymph node metastasis was seen [Table/Fig-3] and the association showed some statistical significance (p-value=0.05).

Vimentin is an intermediate filament normally expressed in mesenchymal cells. Niveditha SR and Bajaj P had reported that the vimentin expression is an indication of biological aggressive tumour [15]. Many authors have reported varied percentage of vimentin expression in malignant cases. Hemalatha A et al., reported 18% and Thomas PA et al., reported 47.1% vimentin positivity in malignant breast lesions in their studies [16,17]. In the present study, 83.6% positivity for vimentin was seen. Hemalatha A et al., Domagala W et al., and Korsching E et al., showed an increase in expression of vimentin with grades which is congruent with the present study. In grade I, 63.6% positivity for vimentin was seen, 82.4% in grade II and 95.5% in grade III tumours [16,18,19]. An increase in expression of vimentin with increase of tumour grade is seen. This association was statistically significant (p-value=0.02).

No significant association was seen between tumour size and vimentin expression in studies done by Niveditha SR and Bajaj P, Hemalatha et al., and Domangala et al., [15,16,18]. There was a positive association between tumour size and expression of vimentin in the present study. Tumour size <2 cm (T1) showed 64.7% expression of vimentin whereas, 85.7% expression was seen in tumour of 2-5 cm size (T2) and 100% expression of vimentin with tumour size >5 cm (T3) was seen and this association was found to be statistically significant (p-value=0.02) [Table/Fig-4].

Vora HH et al., reported that loss of cytokeratin and the gain of vimentin expression are indicators of biologic aggressiveness of the breast carcinoma [20]. Raymond WA and Leong AS had proposed that the knowledge of the list of carcinomas which may be co-expressing vimentin and the cytokeratin might be helpful in the assessment of undifferentiated tumours and metastatic deposits [21]. Thomas PA et al., showed that in their study all breast tumours containing ≤50% keratin expression were immunopositive for vimentin, whereas, only 41% of the tumours with ≥50% keratin expression were vimentin immunopositive [17]. Their results indicate that vimentin immunopositivity in breast cancer tissues is inversely related to keratin expression. The results in present study were in concordance with Vora HH et al., and Thomas PA et al., that loss of cytokeratin expression and a gain in vimentin expression is associated with higher tumour grade and size along with higher chance of metastasis i.e more aggressive behaviour of tumour [17,20].

There has been lot of work to detect cancers and metastasis early and one of the most promising technologies is detecting Circulating Tumour Cells (CTC) which has applications in detecting metastasis, prognostication and to a limited extent in treatment. However, the technology is still under refinement as enrichment of desired cells is a challenge owing to their low concentration but has future implications [22]. In contrast, IHC has been standardised over decades and routinely done even in a resource limited setup and requires much lesser sophisticated equipments and training. Hence, if investigation into EMT is desired, IHC is definitely of choice, if resources are limited. However, it has to be emphasised that multiple markers should be used as some cases can be negative for one or more markers.

Limitation(s)

Sample size and appropriate population representation are the limitations of the study so further studies need to be conducted to firmly confirm the association between the IHC markers and the tumour aggressiveness.

CONCLUSION(S)

In the present study, it was observed that there is down regulation of cytokeratin 5/6 with increase in tumour size, grade and metastasis. An upregulation of vimentin was seen with increase in tumour size, grade and metastasis. Hence, it is inferred that downregulation of cytokeratin 5/6 and upregulation of vimentin can be used to assess EMT which in turn shows higher chances of metastasis. These findings could be used in cases of micro metastasis and doubtful case to assess whether metastasis has occurred or not.

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