Role of E-Cadherin as a Cell Marker in the Differential Diagnosis of Breast Lesions

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Abstract

Aims and Objectives: To diagnose and grade cases of breast cancer and evaluate their relationship to E-cadherin expression and to evaluate the role of E-Cadherin as a diagnostic and prognostic biomarker in Breast Cancer

Material and Methods: 234 patients presenting with signs and symptoms of breast lesions were studied after a detailed history and thorough examination. Mastectomy and lumpectomy were processed for routine histopathology and immunostains for ER, PR, HER2/ NEU and E-cadherin and diagnosed and typed according to WHO classification and grading of malignant cases was done as per Modified Scarff-Bloom-Richardson grading scheme.

Results: Fibroadenoma was the most frequently diagnosed benign lesion, 60 cases (55.6%). All the benign cases showed strong and homogenous membranous positivity with intensity score of 4+. Majority of fibroadenoma cases, 8 (40.0%) showed proportion score 3+. Infiltrating ductal carcinoma (not otherwise specified) was the most frequently diagnosed malignant lesion, comprising 67 cases (53.2%), followed by residual/recurrent ductal carcinoma, in 31 cases (24.6%). Most of the Grade 1 IDC showed 3+ proportion score, with gradually decreasing score in higher grades. Relationship between grade and E-cadherin score showed a p value of 0.0059, which was statistically significant. All the 6 cases (100%) of infiltrating lobular carcinoma were negative for E-cadherin. Medullary carcinoma showed 1 case each of weak 1+ proportion score staining and 2+ Intensity score in <10% of cells.

Conclusions: Expression of E-cadherin is associated with benign or malignant nature of breast lesion, with expression stronger in benign. It can be used as diagnostic modality for subtyping breast cancers as IDC or ILC if morphology is not clear.

Keywords: Breast Carcinoma, Histopathology, Immunohistochemistry.

Introduction

India is moving through a period of dramatic socio economic turnaround. Malignancies are the second most common cause of deaths in India after cardiovascular diseases with a rise in incidence of 0.5%-2% yearly in all age groups but more in younger groups (<45).[1] Cervical cancers have been leading the way, but breast carcinoma has emerged as the most common cancer diagnosed in Urban India, constituting >30% of all cancers in urban females with westernised lifestyle and change in the reproductive behaviour being the main culprit. We are witnessing an age shift. The average age of developing breast cancer has shifted from 50-70 years to 30-50 years; and cancers in the young tend to be more aggressive.[2]

Breast cancer survival rates vary greatly worldwide, ranging from 80% or over in North America, Sweden and Japan, to around 60% in middle-income countries and below 40% in low-income countries.[3] The low survival rates in less developed countries can be explained mainly by the lack of early detection programmes, resulting in a high proportion of women presenting with late-stage disease, as well as by the lack of adequate diagnosis and treatment facilities.

The process of cancer invasion and metastasis consists of a complex series of sequential steps, involving specific tumor cells and host properties.[4] Detachment of tumor cells from the primary lesion is assumed to be the initial and important step in the metastatic process.[5] Coman described that tumor cells are more easily separated from a solid tumor mass than their counterpart normal cells from surrounding tissue.[6] Their detachment is regulated by the property of tumor cell "adhesiveness". However, the molecular basis of the mutual adhesiveness of cancer cells has not been clarified in vivo, and it is difficult to estimate the actual strength of intercellular connection from the expression of a single adhesive molecule.

Recently the existence of abnormal E-Cadherin expression in human cancerous tissues was demonstrated and a significant relationship was found between E-cadherin expression and histological grade or invasiveness in gastric cancer.[7] Proper histopathologic categorization of breast carcinomas has prognostic implications.[8] The majority of invasive lobular carcinomas (ILC) have shown a complete loss of E-cadherin expression. The loss of E-cadherin is from the outset, i.e., in the pre-invasive stage of lobular carcinoma in situ (LCIS). E-cadherin loss explains the histopathologic appearance of LCIS including a diffuse growth pattern of this non-gland-forming tumor with discohesive tumor cells.[9] Keeping in mind the prognostic factors in breast cancer, this study was carried out to diagnose and grade cases of breast cancer and evaluate their relationship to E-cadherin expression and to evaluate the role of E-Cadherin as a diagnostic and prognostic biomarker in Breast Cancer

Material and Methods

The present study was conducted on 234 patients presenting with signs and symptoms of breast lesions attending the Out-patients and In-patients clinics of the Department of Surgery and Pathology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh.

A detailed history, including age of presentation, duration of disease, age at menarche, age at menopause, duration of breast feeding, any first degree relative having positive history for breast cancer, intake of oral contraceptive pills/hormone replacement therapy; complete physical examination and preliminary investigations were recorded in each case and consent taken for surgical intervention.

Mastectomy and lumpectomy specimens with sampled lymph nodes were fixed in 10% neutral buffered formalin, processed for routine histopathology and immunostains for ER, PR, HER2/ NEU and E-cadherin were employed with primary antibody (Novocastra ready to use mouse monoclonal antibodies). H&E stained histological sections were assigned diagnosis and typed according to WHO classification of tumours of the breast (2012) and grading of malignant cases was done as per Modified Scarff-Bloom-Richardson grading scheme.

E-cadherin scoring: 4-point scale[10]: a) Scoring of Intensity: 0 = Negative, 1+ = Weak and heterogeneous, 2+= Mild or weak and homogeneous, 3+= Moderate or strong and heterogeneous, 4+= Intense or strong and homogeneous. b) Scoring of Proportion: 0 = complete absence or negative expression, 1+ = < 10%, 2+ = 10-50%, 3+ = > 50%

Results

Out of a total of 108 benign breast cases, fibroadenoma was the most frequently diagnosed subtype comprising 60 cases (55.6%). This was followed by gynaecomastia in male patients with 12 cases (11.2%); fibrocystic disease and chronic mastitis both 8 (7.4%) cases each; 5 (4.6%) cases of breast abscess; duct ectasia 3 (2.7%) cases and fibroadenomatoid hyperplasia 2 (1.8%) cases. Other histopathological types comprised of 2 cases (1.8%) each of atypical ductal hyperplasia, tubercular mastitis and tubular adenoma and 1 case (0.9%) each of benign phyllodes, rhabdomyoma, hydatid cyst and sebaceous cyst.

Expression of E-cadherin was studied in 20 cases of benign morphology. All of them showed strong and homogenous membranous positivity with intensity score of 4+. Taking into account the proportion of positive cells on E-cadherin staining, majority of fibroadenoma cases, 8 (40.0%) showed proportion score 3+, while 2 (10.0%) cases showed 2+ (Fig. 1). Similarly majority of gynaecomastia cases, 4 (20.0%) showed 3+ proportion score while 1 case showed 2+ score. One case of benign phyllodes showed 2+ positivity and intensity score of 2+. One case each of chronic mastitis and fibrocystic disease showed of 3+ and 2+ proportion score.

Out of the total of 126 malignant breast lesions, infiltrating ductal carcinoma (not otherwise specified) was the most frequently diagnosed lesion, comprising 67 cases (53.2%). This was followed by residual/recurrent ductal carcinoma, 31 cases (24.6%); 7 cases (5.6%) of malignant phyllodes and 6 cases (4.8%) of infiltrating lobular carcinoma. Other histopathological types consisted of 4 cases (3.2 %) of Paget's disease with IDC, 3 cases of tubular carcinoma (2.4%), 2 cases (1.6%) each of medullary carcinoma and mixed ductal and lobular carcinoma and 1 case (0.8%) each of papillary carcinoma, intracystic papillary carcinoma, invasive micropapillary carcinoma and metaplastic carcinoma.

Infiltrating ductal carcinoma showed significant association between grade and expression of E-cadherin. Most of the Grade 1 IDC showed 3+ proportion score, with gradually decreasing score in higher grades. Out of 18 cases of Grade 1 IDC NOS, 12 (66.6%) showed 3+ proportion score, 5 (27.8%) cases showed 2+ score whereas 1 (5.6%) case showed 1+ score (Fig. 2). Out of 15 cases of Grade 2 IDC NOS, 7 (46.7%) showed 3+ and 6 (40%) showed 2+ proportion score with 2 cases (13.3%) showing 1+ score. Out of 10 cases of Grade 3 IDC NOS, 7 (70%) cases showed 2+ proportion score and 3 cases (30%) showed 1+, none showed 3+ score. On applying Fisher Exact probability test between grade and E-cadherin score, p value was 0.0059 which was statistically significant.

In our study, all the 6 cases (100%) of infiltrating lobular carcinoma were negative for E-cadherin (Fig. 3). Medullary carcinoma showed 1 case each of weak 1+ proportion score staining and 2+ Intensity score in <10% of cells. Two cases (100%) of combined ductal and lobular carcinoma of breast showed 2+ proportion score in the ductal portion in both the cases, whereas lobular portion showed complete absence of E-cadherin expression. Two cases (6.4%) out of 31 cases of recurrence of IDC NOS were stained, 1 case showed 2+ proportion score and the other showed 3+ score. One case (100%) of metaplastic carcinoma stained showed 2+ proportion score. Two cases of malignant phyllodes were stained and showed positive membranous staining in epithelial lining in both the cases. The stromal component showed positive cytoplasmic staining in <10% of stromal cells in one case and negative staining in other. Two cases of Paget's disease with IDC NOS, showed strong 3+ proportion scoring of tumor cells in skin as well as in the underlying malignant cells.

Fisher Exact test on E-cadherin scoring on benign and malignant cases showed p value of 0.0169, which was statistically significant, which implied a stronger expression of E-cadherin in benign lesions as compared to malignant.
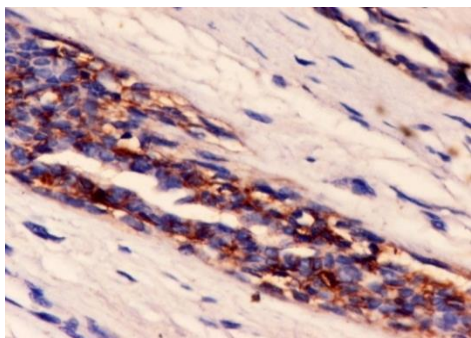
Fig. 1: Fibroadenoma: Photomicrograph showing breast ducts with moderate and heterogenous E-cadherin expression (3+). IHC E-Cadherin x 40X
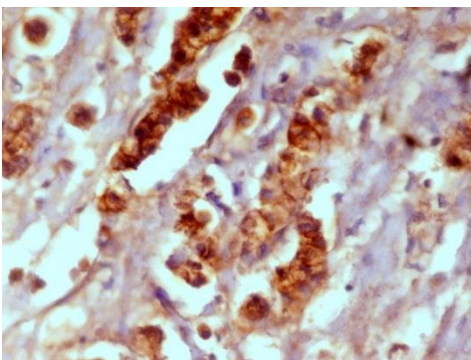

Fig. 2: Infiltrating Ductal Carcinoma Grade 1: Photomicrograph showing strongly positive malignant epithelial cells lining the glands with complete and homogenous membranous staining (4+) in > 50% cells (3+). IHC E-cadherin x 40X
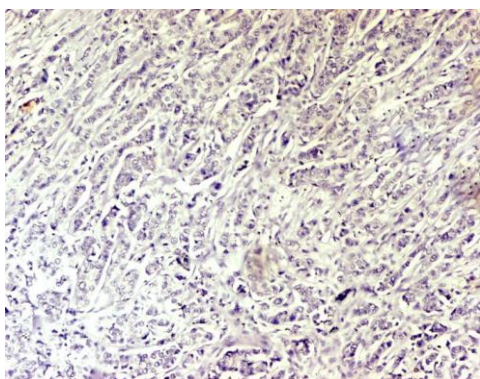

Fig. 3: Infiltrating Lobular Carcinoma shows E-cadherin negativity in tumor cells. IHC E-cadherin x 10X

Discussion

Out of a total of 126 malignant breast lesions, IDC NOS was the most frequently diagnosed lesions, comprising 67 cases (53.2%), followed by residual/recurrent ductal carcinoma 31 cases (24.6%); malignant phyllodes 7 cases (5.6%) and infiltrating lobular carcinoma 6 cases (4.8%). Our results were comparable to studies by Bane et al and Mudholkar et al.[11,12]

Expression of E-cadherin on all the benign lesions showed strong and homogenous membranous positivity with intensity score of 4+, with majority of cases of fibroadenoma, 8 (40.0%) depicting 3+ proportion score. Our findings are consistent with the work of Suciu, who reported strong homogenous E-cadherin positivity in benign lesions of breast.[13]

Infiltrating ductal carcinoma (IDC NOS) showed significant association between grade and expression of E-cadherin. 3+ proportion score was seen mostly in grade 1 cases, with gradually decreasing score in higher grades. A statistically significant finding (p=0.0059) was seen between grade and E-cadherin score in our study. Our findings were consistent with studies of Siitonen, Guriec and Qureshi, who reported that reduced expression of E-cadherin was associated with poor differentiation and higher grade of malignancy.[14-16] Furthermore, recent studies with transfected cell lines have shown that selective loss of E-cadherin expression can generate dedifferentiation and invasiveness of human carcinoma cells.[17]

Recently the existence of abnormal E-cadherin expression in human cancerous tissues was demonstrated with a significant relationship between E-cadherin expression and histological grade or invasiveness in gastric cancer.[7] Schipper et al also suggested that downregulation of E-cadherin expression is associated with dedifferentiation and lymph node metastasis of squamous cell carcinomas of the head and neck.[18] However, the role of E-Cadherin molecules in human cancer has not been clarified by a clinicopathological study with statistical analysis. In order to reveal the correlation of E-cadherin molecules with invasion and metastasis of human cancer cells in vivo, E-cadherin expression in surgically resected human breast cancer tissues and metastatic lymph nodes by means of immunohistochemical staining was studied.

Reduced expression of E-cadherin has been observed in aggressive tumors of the esophagus, ovary, and stomach.[19] Mechanisms by which E-cadherin protein expression is lost include E-cadherin gene mutation and loss of the wild-type allele by loss of heterozygosity, which indicates that E-cadherin is a classic tumor suppressor gene.[20]

However, the practical application of E-cadherin expression in breast cancer as a prognostic and diagnostic cancer biomarker remains controversial. Reduced E-cadherin expression was an adverse prognostic biomarker and associated with high histopathologic grade and nodal metastasis and loss of estrogen receptor (ER) and progesterone receptor (PgR).[11,12] Fisher Exact test on E-cadherin scoring on benign and malignant cases was statistically significant (p=0.0169) in our study, which implied a stronger expression of E-cadherin in benign lesions as compared to malignant, a finding consistent with Suciu et al.[13]

Conclusions

Expression of E-cadherin is associated with benign or malignant nature of breast lesion, with expression stronger in benign. It can be used as diagnostic modality for subtyping breast cancers as IDC or ILC if morphology is not clear. It has a prognostic value in IDC grading and may have an association with Her2/ neu expression which may aid in treatment efficacy.

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