Immunohistochemical Expression of E-cadherin in Invasive Duct Carcinoma of the Breast

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Abstract:

Breast cancer is the most frequent malignant tumor in women worldwide. E-Cadherin is a calcium-dependent epithelial cell adhesion molecule that is expressed at adherens junctions. Its loss may result in a poorly differentiated tumorphenotype. Cells with mutated E-cadherin demonstrate increased motility, altered organization of their actin cytoskeletonand associated with metastases, thereby providing evidence for its role as an invasion suppressor.

Objectives: Assess the prognostic value of E-cadherin expression in invasive duct carcinoma (IDC) of the breastand to evaluate its correlations with other prognostic parameters.

Materials and Methods: Immunohistochemical (IHC) E-Cadherin expression was studied in 60 IDC and the results were correlated with the clinico-pathological parameters.

Results: E-cadherin was positive in 46/60 (76.7%) of IDC; 15/60 (25%) showed strong membranous staining. A significant inverse correlation was found between E-cadherin expression and number of lymph node metastasis.

Conclusions: Loss of E-cadherin expression is a predictive of invasion and metastasis in IDC.

Keywords: Breast cancer,invasive duct carcinoma, E-Cadherin

Abbreviations: IDC: invasive duct carcinoma, IHC: immunohistochemical, PBS: phosphate buffered saline, IHCS:Immunohistochemical scores, PP: percentage of positive cells, staining intensity: SI.Lymphvascular invasion (LVI), Lymph node metastasis (LNM).

Introduction:

Breast cancer is the most common cancer in females all over the world. Its incidence is increasing by 0.5% every year ***(Yang et al., 2018).*** Age incidence tends to decrease nowadays, and morbidity rate is increasing, which severely threatens health of women

around the world ***(Cabioglu, 2016)***. Growing evidence suggested that tumor microenvironment serves a role in the development of cancer and its prognosis ***(Chen et al., 2016).***

Breast cancer consists of glandular epithelial cells from the terminal ductal-lobular unit. Tumor progression and metastasis have different successive stages, such as detachment of tumor cells from primary tumor, attachment to the basal membrane of vascular endothelium with its destruction and releasing of tumor cells into the bloodstream or lymphatic system, and colonization of target organ. During these events, the junction structures between malignant cells, particularly adherens junctions and E-cadherin, have an important role ***(Suciu et al., 2008).***

Cell adhesion molecules are a type of glycoprotein mediating cell-cell and cell-extracellular matrix adhesion, and they serve an important role in the genesis, development, invasion and metastasis of tumors. Decrease in tumor cell adhesion appears to easily affect tumor invasion and metastasis ***(Milioli et al., 2015).***

E-cadherin is a transmembrane calcium-dependant adhesion protein, with molecular weight of 120 kDa, and is part of type I classical cadherin family. It is considered as a tumor suppressor protein ***(Jiang and Mansel, 2000).*** It has been reported that E-cadherin is under-expressed in a variety of tumors, and that it could participate in growth, differentiation, metastasis breast cancer ***(Berman et al., 2013).***

Normal ductal epithelial cells in mammary gland strongly express E-cadherin protein in the cytoplasmic membrane ***(Bukholm et al., 1997).*** Some studies have demonstrated that aberrant E-cadherin expression is associated with high-grade, estrogen receptor negative, and metastatic breast carcinomas, whereas other studies have failed to confirm these findings ***(Moll et al., 1993).***

Based on these data, we investigated the expression pattern of E-cadherin in IDC, and its correlations withtumor size, tumor grade, presence of lymphovascular invasion and lymph nodes metastasis.

Material and Methods:

Tissue samples

Formalin-fixed paraffin-embedded tissue blocks of IDC from 60 patients were enrolled retrospectively from the archive of Pathology and Oncology Department, Qena University Hospital, South Valley University. Approval to perform this work was obtained from the Institutional Research Ethical Committee. Tissue biopsies were 52 cases of radical mastectomy, 3 cases of

simple mastectomy, 3 cases of excisional biopsy, and 2 cases of quadrantectomy. Cinical data of the investigated cases were obtained from patients` clinical files. IDC were stained by H&E and examined to determine the histological type, grade, lymph node status, and lymphovascular invasion.

Immunohistochemistry

A mouse monoclonal antibody of E-cadherin and peroxidase detection system were used. IHC technique was performed according to the manufacturer's protocol but with some modifications as follows: Sections were incubated in xylene, rehydrated by grades of alcohol, immersed in distilled water, then immersed in antigen retrieval solution (pH6) in a microwave (1400W) for 5 minutes, then washed in deionized water. Endogenous peroxidase was neutralized by using peroxidase block; incubated with protein block for 5 minutes; incubated with the primary antibody overnight (dilution 1:100); incubated with secondary antibody for 60 minutes; then with streptavidin for 60 minutes.

Between incubations, sections were washed two times in phosphate buffered saline (PBS) for 5 minutes each. The brown color reaction was developed by using DAB working solution for 5 minutes, counterstained with hematoxylin for 15 minutes, washed in running water, dehydrated and mounted. All steps were performed at room temperature. Normal breast tissue and skin were used as positive control, and negative control was done by omitting the primary antibody.

Interpretation of the results

E-cadherin stain was considered positive when it was membranous or membranous and cytoplasmic according to manufacturer's protocol. A semi-quantitative method according to the following standard score:Percentage of positive tumor cells (PP) was evaluated and scored as: 0 for < 5%, (1) for 5-25%, (2) for 25-50, (3) for 50-75 and 4 for >75. Staining intensity (SI) was considered as (1) for weak, (2) for medium and (3) for intense staining following ***Hussein et al, (2004),*** and ***Baltaziak et al., (2006).***

Immunohistochemical scoring

Immunohistochemical scores (IHCS) were calculated by multiplying the percentage of positive cells; PP with the staining intensity; SI. Hence, the following formula was used; IHCS= PP X SI. Validation of this method has been described elsewhere ***(Damron et al, 2004, and Hussein et al, 2004).***

Statistical analysis

The commercially available statistical software (SPSS version 20) was used for data analysis. Univariate analyses were performed to identify associations between E-cadherin and the studied variables; tumor size, lymph nodes involved by the tumor or not. Each ordinal variable was dichotomized on the basis of number of subjects in various categories. This yielded the following binary values for analysis: E-cadherin; (0 if E-cad= 0, 1+, 1 if E-cad = 2+ or 3+ respectively). Lympho-vascular invasion ; 0 if it is absent, 1 if it is present ***(Van der Auwera et al, 2005).*** Lymph nodes; grade 1 (not affected), grade 2 (one to three affected nodes), or grade 3 (four or more affected nodes) after ***Guerra et al, (2003).***

Results:

This study included 60 specimens of infiltrating ductal carcinomas, The average age of the patients was of 50.68 years, with age rangeof 26-73 years. The average tumor size was 4.09 cm, with range between 1.2 and 10 cm. The majority of tumors were IDC not otherwise specified (NOS)(59 cases), and only one case of medullary carcinoma. Most of the studied cases 53/60 (88.3%) were grade (G) 2, 5/60 (8.3%) of cases were poorly differentiated (G3), and only two cases were G1.

In situ component was present in 13/60 (21.7%) of investigated cases. Lymphovascular, muscle and skin invasion were present in 12/60 (20%), 5/60 (8.33%), 2/60 (3.33%) of the studied cases respectively. Axillary lymph node enlargement was present in 50/ 53 cases of modified radical mastectomy where nodal metastasis was assessed. Nodal metastasis was positive in 28/50 (56%) of the studied cases of IDC, and negative in the remaining cases. Table 1. summarizes histopathological findings of cases of the current study.

E-cadherin was positive in 46/60 (76.7%) of IDC. E-cadherin showed variable degrees of expression in IDC; whereas 15/60 (25%) of cases showed strong membranous staining (Figure 1). Invasive tumors showed variable *in situ* component ranging from 10% to 90% of the tumor tissue. All foci of ductal carcinoma *in situ* showed membranous staining in all cases. A significant association was observed between membranous E-cadherin expression and the number of positive lymph nodes; where 20/22 (90.9%) of node negative cases were E-cadherin positive, only16/28 (57.1%) of the node positive cases were E-cadherin positive ($p<0.01$). However no association was found between E-cadherin expression

and tumor size, tumor grade, or lymphatic vascular invasion.

Table 2. summarizes a multivariable logistic model to predict strong E-cadherin expression and patients' age, tumor size, tumor grades, lymphatic vascular invasion, and number of positive lymph nodes. Table 3. shows E-cadherin expression in IDC in relation to tumor grade.

E-cadherin expression is a predictor of negative nodal metastasisin breast cancer cases while absence of E-cadherin expression is a predictor of positive nodal metastasis in the studied cases ($p<0.01$). Table 4. showed E-cadherin expression in IBC in relation to lymph node status.

Table 1. Histopathological findings

Parameter	**No. of cases**
Histological type(60cases)	
IDC NOS	59(98.3%)
Medullary	1(1.7%)
Tumor grade (60cases)	
Grade I	2(3.33%)
Grade II	53(88.33%)
Grade III	5(8.33%)
LVI (60cases)	
Absent	48(80%)
Present	12(20%)
LNM (50 cases)	
Absent	22(44%)
Present	28(56%)

Lymphovascular invasion (LVI),
Lymph node metastasis (LNM)

Table 2. E-cadherin expression in relation to clinico-pathological variables

Parameter	E cadherin		P value
	Negative	Positive	
Age			
<45	9(45%)	11(55%)	0.005
≥45	5(12.5%)	35(87.5%)	
Tumor size			
<2cm	2(28.6%)	5(71.4%)	0.496
2-5cm	8(20.5%)	31(79.5%)	
≥5cm	4(28.6%)	10(71.4%)	
Tumor grade			
GI	2(3.3%)	0(0%)	0.187
GII	11(20.8%)	42(79.2%)	
GIII	1(20%)	4(80%)	
LVI			
Absent (0)	11(22.9%)	37(77.1%)	0.879
Present (1)	3(25%)	9(75%)	
LNI (No)			
0	2(9.1%)	20(90.9%)	
1	3(75%)	1(25%)	0.0001
2	9(37.5%)	15(62.5%)	
3			

Table 3. E-cadherin expression in IDC in relation to tumor grade

Tumor grade	**E-cadherin expression (IHCS)**									**IHCS (X±SD)**
	Mild			**Moderate**			**Marked**			
	0	1	2	3	4	6	8	9	12	
Grade I (2)	1	0	1	0	0	0	0	0	0	1±1.41
Grade II (53)	11	2	7	7	2	6	2	8	8	5±4.26
Grade III (5)	2	0	0	1	0	0	0	0	2	5.4±6.14
P value	0.439									

P value is significant at < 0.05.

Table 4. E-cadherin expression in IDC in relation to lymph node status

Lymph Node	E-cadherin	
	Low(23)	High(27)
Negative(22)	6	16
Positive(28)	17	11
P value	0.01	

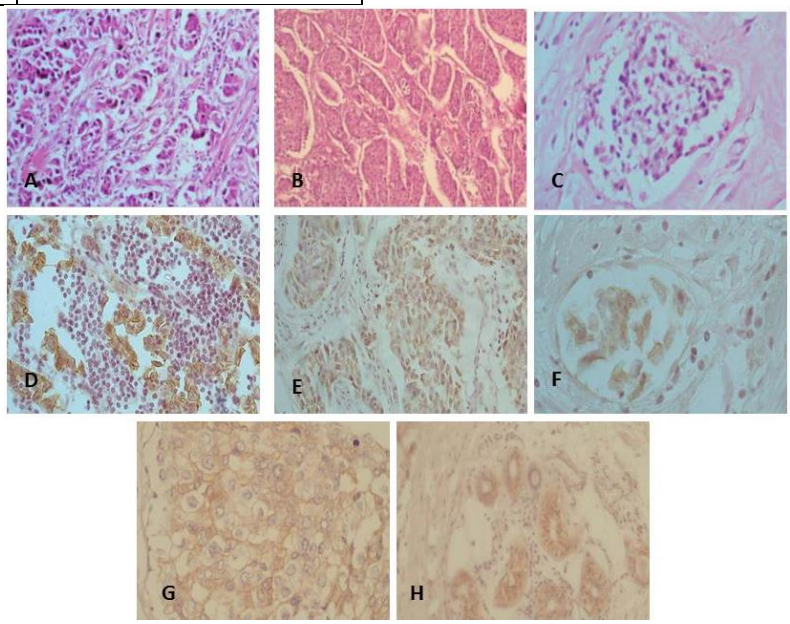

Figure (1): A: IDC, H&E (x200), **B:** Medullary carcinoma H&E (x100), **C:**Vascular invasion in a case of IDC, **D:** Stromal and inflammatory cells were negative for E-cadherin (x200), **E:** Normal breast tissue showing strong membraneousE-cadherin expression (x200), **F:** E-cadherin expression in vascular embolus in a case of IDC (x200), **G:**IDC showing strong membraneous staining for E-cadherin (x200), **H:** IDC showing strong membraneous staining with E-cadherin (x400).

Discussion:

In normal epithelial tissues, proliferating epithelial cells are controlled by a mechanism called contact inhibition, it means that when the density of proliferating cells reaches a certain value, growing and proliferation rate decreases. In tumor cells this contact inhibition will be lost due to abnormal adhesion molecules like E-cadherin **(Suciu et al., 2008).** Changes in adhesion molecules may reduce adhesion of tumor cells, contributing to tumor infiltration and metastasis. Therefore, changes in cell adhesion are the main mechanism of invasion and metastasis. Breast cancer metastasis seriously affects prognosis ***(Yang et al., 2018).***

In the present study, E-cadherin expression was positive in 76.7% of IDC; staining was strong linear at the cell borders of well and moderately differentiated tumors but was heterogeneous and dotted over cell borders in high grade tumors. Our results are close to those of ***Moll et al., (1993), Howard et al., (2005), and Younis et al., (2007)*** which showed that E-cadherin positivity in IDC was present in 79%, 84% and 72% of cases respectively.

The present study results suggested that low expression or deletion of E-cadherin was positively associated with lymph node metastasis, and with number of positive lymph nodes. Negative expression rate was significantly higher in breast cancer patients with local lymph node metastasis than in patients without local lymph node metastasis, and higher in patients with larger number of lymph node metastasis, and these differences were statistically significant (p=0.01& 0.0001 respectively).This result is similar to the findings of ***Banklavi et al., (1999)***, ***Younis et al., (2007) and Yang et al., (2018)*** whoreported that node negative tumors had associated with strong E-cadherin expression.In contrast, ***Howard et al., (2004)*** found persistence of strong expression in cases with more lymph node positivity and proposed that increased expression of E-cadherin is necessary for tumor progression in patients with aggressive breast cancer. The disparity between the results of different studies may be due to differences in investigated patients, which might be indicative of disparity in biology of breast cancer in divergent populations and also variation in histological types of populations in different studies **(*Younis et al., 2007*).**

Persistence of E-cadherin expression in high grade tumors, large sized tumors, and tumors with lymphovascular invasion

contrasts with most of the reports of E-cadherin in breast cancer which have described down-regulation of this molecule in tumorigenesis. The significance of its expression is unclear at this point. These results are somehow in contradiction with the literature data regarding involvement of E-cadherin in tumor metastases. One explanation is that in these cases the level of cadherin may be normal, but not from the functional point of view. Another scenario is that only the complete E-cadherin/catenin complex is associated with no evidence of metastasis. Altering of the cadherin/catenin complex without altering cadherin expression may be an explanation for tumor progression and metastases ***(Suciu et al., 2008).***

Understanding these mechanisms and further investigating the findings of this study will aid in confirming these results. Further research is required to adequately understand the decrease in cell adhesion.

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