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Immunohistochemical Detection of Laminin Protein in Sudanese Women with Breast Tumors

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Abstract*: This is hospital based descriptive retrospective case study aimed to detect the expression of laminin in breast tumors using immunohistochemical method. Forty paraffin embedded blocks from patients samples previously diagnosed as breast tumors were collected. One section of 3 micron thickness was cut from each paraffin block by rotary microtome and stained by immunohistochemical method for detection of laminin. Data collected from patient's files and results were analyzed using SPSS computer program. Samples included 20 malignant tumors, including invasive ductal carcinoma 16 samples, lobular carcinoma 2 samples, micro papillary carcinoma one sample and metaplastic squamous cell carcinoma one sample. And 20 samples were benign tumors; all of them were fibroadenoma. Grade of malignant tumors were one sample was grade 1, 9 samples were grade II, 6 samples were grade III, 4 samples were not graded. The patients age range between 18 to 90 years with mean age of 40 years. Malignant breast tumors showed strong positive expression of laminin in 7 samples, and weak positive in 13 samples, while all benign tumors showed strong positive expression of laminin with significant association (P=0.000). This study concluded that breast tumor tissue express laminin with high expression in benign tumors.*

Keywords— Laminin; breast tumors; Immunohistochemistry.

1. INTRODUCTION

Breast tumors usually start from the ductal hyper proliferation, and then develop into benign or even metastatic carcinomas after constantly stimulation by carcinogenic factors [(1)]. It is the main cause of cancer-related death in women in developing countries and second leading cause in women in developed countries [(2)].

Laminins (LMs) are abundant extracellular matrix (ECM) proteins present predominantly in basement membranes (BM). At least 16 isoforms have been described and named according to their specific trimeric combination of a, β and c chains using the new nomenclature [(3)].

Laminin expression has been implicated in the hallmarks of carcinogenesis; including cell proliferation, invasion metastases and the epithelial-mesenchymal transition [(4)].

Laminin is involved in breast cancer invasion and metastasis, and can use this to determine whether the integrity of a basement membrane for differential diagnosis of benign and malignant breast tumors [(5)].

Positive expression of laminin 332 was identified in the tumor cells of 56 cases (70%) of the 80 TN cases; expression was identified in only 15.2% of the non-TN cases [(6)].

In 887 cases of primary breast carcinoma tested, 244 (28%) were found to be positive in the cytoplasm, with a positivity ranging from 30% to 100% of tumor cells [(7)].

2. Materials and methods:

2.1 Materials:

Archived tissue blocks obtained from samples breast tumors were used in this study.

2.2 Methods:

2. 2.1 Study design:

This is a hospital based descriptive retrospective case study aimed to detect laminin expression in breast tumor using immunohistochemical method.

2.2.2 Sample processing:

Section to be stained were cut at 3µm thickness by rotary microtome, mounted in positively charged glass slides and put at 60˚C oven for 30 minutes

2.2.2.1 Immunohistochemical staining:

The section of 3µm thickness were obtained from formalin fixed paraffin embedded tissue using a rotary microtome, then immunostained using monoclonal antibodies by new indirect technique as follows:

Sections were dewaxed in hot oven and cleared in two changes of xylene for two minutes, then hydrated through descending concentrations of ethanol (100%, 90%, 70%, 50%) and water two minutes for each, then Ag retrieval by water bath retrieval technique for thirty minutes at 97°c (coplin jar containing citrate buffer pH 6.0), then washed in phosphate buffer saline (pH 7.4) for five minutes, then section use circulated by Dako pen, then treated with hydrogen peroxide solution for fifteen minutes, then washed in phosphate buffered saline (pH 7.4) for five minutes, then treated with anti laminin primary antibody for thirty minutes, then rinsed in phosphate buffered saline (pH 7.4), then treated with secondary polymer conjugated antibody for thirty minutes, then rinsed in phosphate buffer saline (pH 7.4), then treated with DAB for seven minutes, then washed in phosphate buffer saline (pH 7.4) for five minutes, then counter stained in Mayer's haematoxylin for one minutes, then washed and blued in 0.05% ammoniated water for 16 second, then washed in tap water, then dehydrated through

ascending concentrations of ethanol (50%, 70%, 90%, 100%), then cleared in xylene and mounted in DPX mountant [8].

2.2.3 Result interpretation:

All quality control measures were adopted during sample staining and immunohistochemical results assessment. Positive and negative controls were used to confirm location of positivity of laminin expression that was confirmed by five cells per one filed. All quality control measures were adopted; positive and negative control slides were used during immunohistochemical staining.

2.2.4 Data analysis :

Data analysis was done using SPSS 11.5 computer program. Frequencies mean and chi-square test values were calculated.

2.2.5 Ethical consideration:

Sample collected after taking ethical acceptance from hospital administration

RESULTS

A total of 40 samples collected from patients samples affected with breast tumors were investigated, 20(50%) of them were malignant tumors, including invasive ductal carcinoma 16(40%) samples, lobular carcinoma 2 (5%) samples, micro papillary carcinoma 1 (2.5%) sample, metaplastic squamous cell carcinoma 1 (2.5%) sample, the remaining were benign tumors samples all of them were fibroadenoma 20 (50%) samples, as indicated in table (4.1). The description of cancer grade revealed that 1 (5%) samples were grade 1, 9 (45%) samples were grade 11, 6 (30%) samples were grade 111, 4 (20%) samples were not graded, as indicated in table (4.2). The age of study population showed that 40 and less years were 20 (50%) patients and more than 40 years were 20 (50%) patients, as indicated in table (4.3). Malignant breast cancer revealed strong positive expression of laminin in 7(35%) samples and weak positive expression in 13 (65%) samples, while all benign tumors showed strong positive expression of laminin , this result showed significant association (P.value=0.000), as indicated in table (4.4).

Table (1): Histopathological diagnosis of the study samples:

Histopathology diagnosis	Frequency	Percent%
Invasive ductal carcinoma	16	40
Lobular carcinoma	2	5
Micro papillary carcinoma	1	2.5
Metaplastic squamous cell carcinoma	1	2.5
Fibroadenoma	20	50
Total	40	100

Table (2): Distribution of cancer grade among malignant breast tumors:

Tumor grade	Frequency	Percent%
Grade I	1	5
Grade II	9	45
Grade III	6	30
Not graded	4	20
Total	20	100

Table (3): Distribution of age group among study population:

Age group (year)	Frequency	Percent%
40 and less	20	50
More than 40	20	50
Total	40	100

Table (4.4): Relation between laminin expression and histopathological diagnosis of breast tumors:

Histopathology diagnosis	Laminin expression		P.value
	Strong Positive	Weak positive	
Malignant	7	13	0.000
Benign	20	0	

DISCUSSION

In this study forty samples from patients affected with breast tumor were investigated by immunohistochemical method for detection of laminin expression. The study revealed that the age of study population range from 18 to 90 years with mean age of 40 years. Most malignant type patients were more than 50 years; this is due to the exposure to carcinogens over a longer period oftime and the decreasing power of the immune system with age. This result was agreed with Sun *et al.* [1], who reported that the incidence ofbreast cancer is highly related to the increasing age. Also compatible with result observed by Shah *et al.* [9], who reported that the risk of developing breast cancer increases with age. This result was disagree with Parsa *et al.* [10], who reported that breast cancer occurred before the age of 40 is clinically more aggressive and has a higher possibility of metastasis and lower survival of older patients.

Most type from malignant samples founded was invasive ductal carcinoma; this result agreed with Zangouri *et al.* [11], who reported that invasive ductal carcinoma was the most common subtype of breast carcinoma and responsible for significant breast cancer mortality.

Laminins are large extracellular glycoprotein's that are expressed by basal epithelium and areimportant components of basement membranes that enhances themigration and invasion of breast carcinoma cells. In this study strong expression of laminin is observed in malignant breast tumors 7/20, and all benign breast tumors showed strong expression also. This relation showed significant association (P.value=0.000), this finding is compatible with result observed by Aoj *et al.* [4], who reported that laminin was expressed in 146 (57.3%) cases wereconsidered positive for laminin expression. Also compatible with result observed by Pellegrini *et al.*[7], who reported that of 887 cases of primary breast carcinoma tested, 244 (28%) were found to be positive in the cytoplasm, with a positivity ranging from 30% to 100% of tumor cells.

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