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Original Article

Pathology Section

Laminin 332: A New Hope as a Prognostic Marker in Triple Negative Breast Carcinomas

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ABSTRACT

Introduction: Laminin expression is supposed to be associated with a number of invasive carcinomas, particularly squamous cell carcinoma of the oral cavity and uterine cervix. Overexpression of laminin is seen in a high proportion of triple negative breast carcinomas, which is associated with an unfavourable clinicopathological state and poor prognosis.

Aim: To evaluate the role of laminin 332 expression as a prognostic marker in triple negative breast carcinomas.

Materials and Methods: This prospective study was conducted in the Department of Pathology, SCB Medical College, Cuttack, Odisha, India, during the period from January 2018 to December 2020. Total number of histologically diagnosed breast carcinoma cases reported was 322, over a span of two years. Out of which, 59 cases showed Estrogen Receptor (ER), Progesterone Receptor (PR) and Human Epidermal growth Receptor 2 neu (HER2neu) negativity. These triple negative breast carcinoma cases were further subjected to laminin immunostaining, excluding three due to lack of sufficient tissue. The results were interpreted and represented in a tabular manner taking into account the clinicopathological manifestation, tumour grading and correlation with laminin expression.

Results: Only the 56 triple negative breast carcinomas were subjected to laminin immunostaining. Majority 50 (89.28%) were elderly patients above 50 years of age. Also, a higher proportion of cases i.e. 36 (64.29%) were grade 3 tumours. Histopathologically, 55 (98.21%) cases were Infiltrating Duct Carcinomas (IDC). Lymph node metastasis and lymphovascular invasion were found in 32 (57.14%) and in 36 (64.29%) cases, respectively. Laminin was strongly positive in those with higher grade (grade-3) tumours 24 (66.67%), those with lymph node involvement 17 (53.12%) and those showing lymphovascular invasions 28 (77.78%).

Conclusion: Laminin immunostaining is an important prognostic marker in a number of epithelial malignancies. The present study was intended to predict the role of laminin as a prognostic biomarker in the triple negative breast carcinoma cases.

Keywords: Basement membrane, Breast neoplasms, Immunohistochemistry

INTRODUCTION

Laminin 332, a major basement membrane component, has a more complex role in cell migration and tumour invasion. Epithelial malignancies showing laminin 332 expression have been shown to correlate well with tumour invasiveness and poor prognosis. It seems to be associated with squamous cell carcinomas, prostatic cancers, melanoma, colonic carcinoma, breast, pancreas and lung cancers [1]. Laminin 332 is expressed at the interface of the tumour with the surrounding stroma and in the cytoplasm of tumour cells. Besides a few exceptions, basal cell carcinoma, advanced breast cancer and prostatic cancer show high levels of laminin expression [2]. Among all breast cancers, triple negative breast cancer is considered to be more aggressive and have a poorer prognosis. These carcinomas are defined by a lack of expression of ER, PR and HER2neu gene expression. Many of these cases possess a basal-like phenotype in which laminin 332 is primarily overexpressed [3]. Studies have shown that triple negative breast cancer is more likely to have distant spread and tends to recur after treatment. Immunohistochemical studies of laminin 332 expression in mammary carcinoma reveals that, around 70% of triple negative breast cancers are positive for laminin 332. Also, because laminin associated interactions take place extracellularly, so targeted antibody intervention can be a mode of cancer therapy. The present study will guide the clinician to assess the aggressiveness of the tumour, plan treatment strategy and also administer targeted therapy.

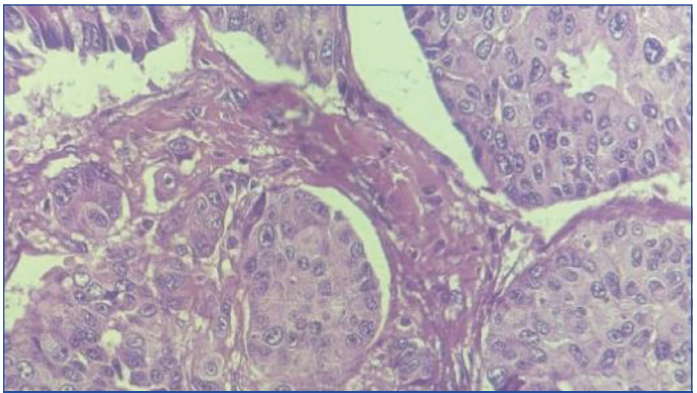
Objectives of the present study was to evaluate laminin 332 expression as a valuable marker in predicting prognosis in triple negative breast carcinomas as well as, comparing with different clinicopathological parameters.

MATERIALS AND METHODS

This was a prospective study, conducted in the Department of Pathology, SCB Medical college, Cuttack, Odisha, India during the period from January 2018 to December 2020. The present study was approved by the Institutional Ethical Committee (IEC) (No. 312) of the Institution. Written informed consent was obtained in each case.

Inclusion criteria: Histopathologically confirmed duct carcinoma of breast [Table/Fig-1] and one case of medullary carcinoma, immunohistochemically confirmed cases of triple negative (ER, PR, HER2 negative) breast carcinomas, presence of adequate tumour tissue with sufficient connective tissue stroma were included in the study.

Exclusion criteria: Insufficient tissue, tissue with inconclusive diagnosis, cases undergone surgery, chemotherapy or radiotherapy in the past were excluded from the study.



[Table/Fig-1]: Microphotograph showing features of duct carcinoma of breast (H&E, 40X).

Serial number of the received specimens, detailed clinical history and presentation, clinical diagnosis, location of the tumour, lymph node status, histopathological diagnosis, tumour grading and staging (based on the modified Scraff Bloom Richardson grade system) was done and the data was entered in the excel sheet. A total number of 322 invasive duct carcinomas of breast and a single case of medullary carcinoma of breast were studied. All these cases were subjected to ER, PR and HER2neu immunostaining. Out of which, 59 cases were reported to be triple negative. Amongst the 59 triple negative cases, 56 cases {55 (98.21%) cases of IDC and only 1 (1.78%) case of medullary carcinoma} were subjected to laminin immunostaining and three cases were excluded, due to lack of tissue samples.

Laminin Immunostaining

Tissue sections, 3 µm thick were mounted on poly-lysine coated slides and incubated at 37°C overnight. The slides were deparaffinised in xylene and rehydrated through graded proportions of alcohol, brought upto water level and subjected to antigen retrieval in microwave oven, two cycles at 96°C for six minutes. The tissues were then cooled to room temperature and incubated with peroxide block for 12 minutes for blocking endogenous peroxidase activity. Then treated with protein block for 10 minutes to eliminate background staining. Subsequently, the sections were incubated with primary antibody (10% goat serum) for two hours, followed by secondary antibody (1:400 dilutions of mouse antihuman laminin monoclonal antibody) for 30 minutes. The slides were then incubated with Novolink polymer for 30 minutes and finally with freshly prepared 3, 3'-diaminobenzidine (DAB) chromogen (1 in 20 ratio) for 1-2 minutes. Finally, the slides were washed in water to remove excess DAB and counter stained with Mayer's Haematoxylin, dehydrated, cleared and mounted with DPX. Oral mucosa was taken as control.

Interpretation of Staining

Ten consecutive representative fields were examined in both 10X and 40X in each case included in the study and compared with the internal control and scored. Presence of brown-coloured end product was indicative of positive immunoreactivity. Normal breast tissue showed a positive expression of laminin in the basement membrane around mammary glands as a continuous linear staining. But in IDC, laminin was strongly positive, irregularly distributed with loss of continuity. Basement membrane of the epithelium, blood vessels, nerves and muscles were taken as internal positive control. In the tumour part, distribution of the stain was searched for around the basement membrane of malignant epithelial cell nests along with its continuity and also within the cytoplasm of the malignant cells. Both the area of staining and staining intensity was evaluated for obtaining accurate results. The rates of expression of laminin were observed using a semi-quantitative 4-tier system of intensity classification (0, 1+/weak staining (light yellow); 2+/moderate staining (yellow/brown); 3+/strong staining (brown)) in a semi-quantitative way and was graded as grade I, II and III respectively. Laminin 332 expression was exclusively cytoplasmic in all the positive cases. It showed strong positivity (3+) at tumour borders [4].

STATISTICAL ANALYSIS

The results of immunohistochemical staining were noted down in tabular form. The number of cases in each category were also expressed in the form of percentages. The whole data was analysed using Statistical Package for Social Sciences (SPSS) version 21.0 software. Chi-square was used to test for association between categorical variables (laminin biomarker expression, histopathological subtypes, tumour grade etc.). The categorical variables were compared to find out the association between the different variables. A value of p<0.05 was taken as statistically significant.

RESULTS

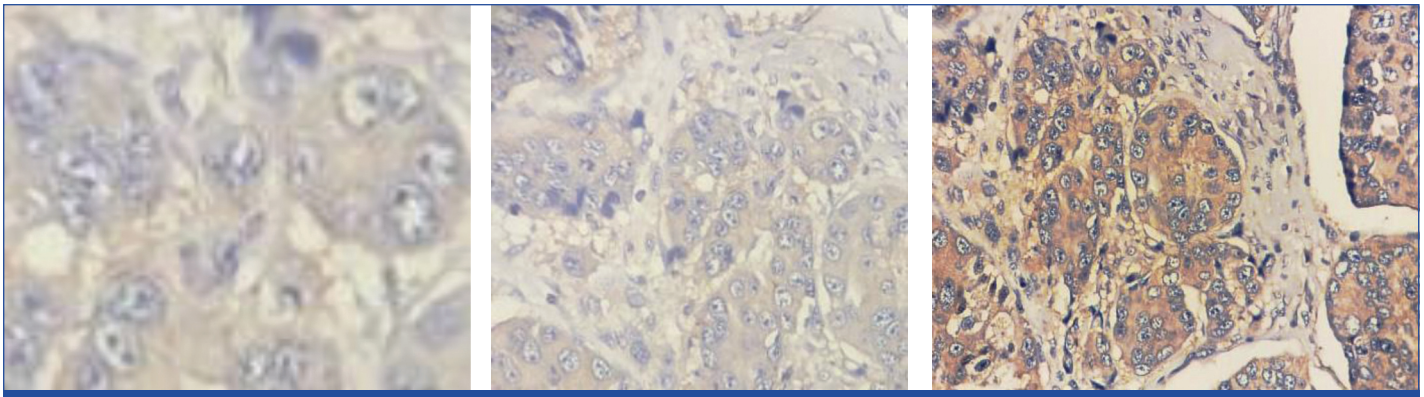
Out of the total number of 56 triple negative breast carcinoma cases subjected to laminin immunostaining, 30 (53.57%) cases showed laminin positivity and 26 (46.43%) cases were negative for laminin. A total number of 50 (89.29%) cases presented at an advanced age and with a higher grade 36 (64.29%) tumour. The average age group was 55 years. Most of the cases 50 (89.28%) were postmenopausal, above 50 years of age) and only 6 (10.71%) cases were below 50 years of age. Out of the total 56 triple negative breast carcinomas studied, lymph node involvement was found in 32 (57.14%) cases. Lymphovascular invasion was found in 36 (64.29%) cases.

Expression of Laminin in Triple Negative Breast Carcinoma

Laminin 332 was positive in 28 (56%) cases in patients above 50 years of age, in contrast to only 2 (33.33%) laminin positive cases below 50 years of age which was not statistically significant (p-value >0.05). Majority, 33 (60%) cases of IDC showed laminin positivity, whereas the single case of medullary carcinoma breast was laminin negative, which was not statistically significant (p-value >0.05). Majority of the tumours, 26 (86.67%) cases with size more than cm centimetre showed laminin positivity, statistically significant (p-value <0.001). Similarly, laminin was positive in majority 24 (66.67%) cases of higher grade (grade-3) tumours, statistically not significant (p-value >0.05), equivocal in grade I and laminin negative staining was more 9 (56.25%) cases of the intermediate grade (grade-2). Out of the total 32 lymph node involved cases, 17 (53.13%) cases showed laminin positivity, statistically significant (p-value <0.05). Also, out of total 36 (64.29%) cases showing lymphovascular invasion, 28 (77.78%) cases showed strong laminin positivity which was statistically significant (p-value <0.001) [Table/Fig-2]. Laminin 332 showed an exclusive cytoplasmic expression in all positive cases [Table/Fig-3-5]. High grade tumours with lymph node involvement and lymphovascular invasion showed strong laminin positivity. This implies laminin 332 is expressed more in high grade tumours.

Parameters	N=56, N (%)	Laminin-Negative, n=26	Laminin-Positive, n=30	p-value
Age (years)				
<50	6 (10.71)	4 (66.67)	2 (33.33)	>0.05
>50	50 (89.29)	22 (44)	28 (56)	
Tumour size (n=56)				
T1 (<2 cm)	10 (17.86)	8 (80)	2 (20)	<0.001
T2 (>2 cm and <5 cm)	16 (28.57)	12 (75)	4 (25)	
T3 (>5 cm)	30 (53.57)	4 (13.33)	26 (86.67)	
Tumour grade				
Grade-1	4 (7.14)	2 (50)	2 (50)	>0.05
Grade-2	16 (28.57)	9 (56.25)	7 (43.75)	
Grade-3	36 (64.29)	12 (33.33)	24 (66.67)	
Lymph node involvement				
Positive	32 (57.14)	15 (46.87)	17 (53.13)	<0.05
Negative	24 (42.86)	4 (16.67)	20 (83.33)	
Histopathological subtype				
IDC NOS	55 (98.21)	22 (40)	33 (60)	>0.05
Medullary	1 (1.79)	1 (100)	0	
Lymphovascular invasion				
Present	36 (64.29)	8 (22.22)	28 (77.78)	<0.001
Absent	20 (35.71)	18 (90)	2 (10)	

[Table/Fig-2]: Laminin immunoreactivity and the clinicopathological relation in the 56 triple negative breast carcinoma cases along with the results of Chi-square test.
IDC: Infiltrating duct carcinoma; NOS: Not otherwise specified



[Table/Fig-3]: Microphotograph showing laminin 332 immunostaining of mild intensity 40X. **[Table/Fig-4]:** Microphotograph showing laminin 332 immunostaining of moderate intensity 40X. **[Table/Fig-5]:** Microphotograph showing intense laminin 332 immunostaining at tumour border and within the tumour cell cytoplasm. (Original magnification 200X). (Images from left to right)

DISCUSSION

Triple negative breast carcinomas, particularly of basal cell type show an aggressive behaviour. Newer diagnostic molecules like laminin acts as an important prognostic marker. In non neoplastic breast, laminin staining is seen along basement membranes around ducts and lobules, as well as, blood vessels as regular continuous linear structures with no intracellular staining. But in inraductal and invasive duct carcinomas, it takes an irregular, thickened or dark staining pattern at the malignant epithelial cell stroma interface [5]. In IDC, the tumour cells grow along with the stroma, without intervening basement membrane. But the laminin staining around invasive groups or sheets of tumour cells indicates that there exists some basement membrane material, possibly secreted by the tumour cells. This pattern of laminin immunostaining also suggests that both in situ and invasive breast carcinomas possess variable abilities to produce laminin. Since basement membranes are produced only along the interface between the epithelial cells and interstitium, the stroma appears to be necessary for basement membrane synthesis.The presence of laminin staining in IDC indicates that these tumours contain basement membrane structures, not easily detectable by routine Haematoxylin and Eosin (H&E) staining. Use of both mono and polyclonal antibodies, show that laminin deposition is predominately extracellular. Laminin 332 is highly expressed in several types of epithelial tumours, where it accumulates at the interface of the tumour with the stroma, reminiscent of its basal location in normal breast [6]. Breast carcinomas with various histologic types and degree of differentiation show a heterogeneous pattern of laminin distribution in both in situ and invasive components, indicating that only some tumour cells are able to produce the basement membrane material. These cells lie adjacent to the stroma, which appears to be necessary for basement membrane synthesis. In the present study, a total number of 322 cases of infiltrating breast carcinomas were studied. Tumour grading and staging were done based on the modified Scraff Bloom Richardson Grade system [7]. These cases were then subjected to ER, PR, HER2neu immunostaining, out of which 59 cases showed triple negativity. But three cases were excluded from the study due to lack of sufficient tissue. Out of the 56 cases, of triple negative breast carcinoma cases studied, 50 (89.29%) cases were above the age of 50 years and only 6 (10.71%) cases were premenopausal below 50 years of age. This finding correlated with that of Colette C et al., where out of 98 cases studied, 61 (62.24%) cases were above 50 years of age and 37 (37.75%) cases were in the premenopausal age group below 50 years of age [8]. The rates of laminin expression were scored in a semi-quantitative four-tier system and graded as grade I, II and III. Laminin expression was found to be associated with adverse clinical, pathological and prognostic features. The greater the tumour size, grade, lymph node involvement and lymphovascular invasion, more intense was the laminin expression. These findings were comparable with those of Agboola AOJ et al., [9]. Laminin 332 stimulates the migration of breast and other carcinoma cells and correlates with tumour-invasiveness [10]. Dysregulated expression of laminin 332 in cancer switches it to a protein favouring migration and tumourigenesis. Also, the laminin gamma 2 chain is highly expressed in invasive mammary cancer cells. Present study revealed expression of laminin 332 in a high proportion of triple negative breast cancers, accounting for 24 (66.67%) of cases of the high grade (grade-3) tumours, comparable to Marica SG et al., Kwon SY et al., examined the expression of laminin 332 in 80 triple negative breast carcinoma cases and found a similar results of 70% immunostaining [4,11]. The cytoplasmic laminin was taken into consideration as it was more likely to be a product of the tumour itself, rather than a secretory protein of stromal cells or residual basal epithelium. Early-stage breast carcinomas showed a reduced, but detectable levels of laminin expression than advanced tumours. Also, the alpha 3 and beta 3 chains of laminin 332 tend to be reduced, whereas gamma 2 levels are elevated in advanced breast carcinomas. Giannelli G et al., showed a strong association of occurrence and metastasis in hepatocellular carcinomas expressing laminin 332 gamma 2 chain [12]. Tsuruta D et al.,showed, co-expression of laminin 332 and its receptors might result in an autocrine stimulation of migration of tumour cells and metastasis in breast carcinoma cases [13]. Several studies have postulated that extracellular matrix protein is overexpressed in epithelial malignancies. This is probably due to accumulation of mutated proteins that modulate signal transduction pathway and thereby, promote cell migration, invasion and metastasis [14]. The expression of gamma 2 chain of laminin is associated with the infiltrative pattern of mucinous ovarian neoplasms [15]. Shinichiro T et al., reported that the high intensity of cytoplasmic expression of laminin gamma 2 chain correlates with high invasive potential, distant metastasis and a poor prognosis [16]. Several experimental studies have shown the impact of laminin 332 expression in cancer progression [17].

Limitation(s)

Limitations of the study were complete medical history like (involvement of distant lymph nodes or other body organs), treatment information was not available for the entire group of cases.

CONCLUSION(S)

Triple negative breast carcinomas show aggressive behaviour, with poor clinicopathological outcomes. Laminin immunostaing can be used as a potential prognostic marker in predicting the outcomes of these triple negative breast carcinoma cases. Also, using laminin antibodies as an effective chemotherapeutic agent, appropriate cancer management can be done and patient survival be improved.

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