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ORIGINAL ARTICLE

Evaluation of Laminin Expression in Invasive Breast Carcinoma and Its Relationship with Clinicopathological Parameters

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ABSTRACT

Breast cancer progression is significantly influenced by extracellular matrix dynamics. Laminin, a key structural glycoprotein, plays a pivotal role in regulating cell adhesion, migration, and metastasis. This study aims to evaluate laminin expression in invasive breast carcinoma and its relationship with clinicopathological parameters. A prospective cross-sectional study was conducted on 53 cases of invasive breast carcinoma. Relevant clinicopathological data were documented, and formalin-fixed paraffin-embedded tissue sections were evaluated for laminin expression using manual immunohistochemical staining. The cohort predominantly comprised females with a mean age of 51.2 years, where invasive ductal carcinoma of no special type (NST) was the most common subtype. Laminin immunoreactivity was detected in 69.8% of the cases. Statistical analyses demonstrated that laminin positivity was significantly associated with older patient age (>50 years), advanced histological grade (grade 3), larger tumor size (>2 cm), and the presence of lymph node metastasis. No significant associations were detected regarding histological subtype, lymphovascular invasion, or perineural invasion. The findings suggest that laminin immunoreactivity is associated with several clinicopathological features of aggressive breast carcinoma. Its association with adverse clinicopathological features suggests a potential role for laminin immunoreactivity as a marker of tumor aggressiveness; however, further studies are needed to establish its independent marker of tumor aggressiveness and adverse clinicopathological features. While laminin immunoreactivity is associated with adverse clinicopathological features, further large-scale, longitudinal studies incorporating survival data and multivariate analysis are required before considering its routine integration into breast cancer diagnostics.

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1. INTRODUCTION

Breast cancer represents the most frequent cause of cancer diagnosis among women globally, with marked heterogeneity across regions [1]. By 2020, these figures had risen to approximately 2.3 million cases and 685,000 deaths, making it the most frequently diagnosed cancer globally and the fifth leading cause of overall cancer mortality [2]. Robust prognostic parameters that allow for predicting the course of disease include the age of patients, proliferation index (Ki-67), tumor grade and stage, hormone receptor status, HER2 overexpression, and

lymph node involvement [3]. Breast cancer progression is mediated not only by intrinsic tumor cell genetic and molecular changes, but also by extensive rewiring of the extracellular matrix (ECM), which critically modulates tumor growth, invasion, and metastatic dissemination through biochemical and biomechanical regulation of human malignant cells [4]. Tumor invasion and metastasis are multistep processes involving tumor growth, local proteolysis, and migration of tumor cells through the degraded ECM. These steps require dynamic interactions between tumor cells and the ECM [5]. Among them, laminin, a key structural component of the basement membrane, plays a crucial role in ECM-mediated signaling. Laminin is a large family of high molecular weight extracellular matrix glycoproteins that assemble as heterotrimers composed of disulfide-linked α, β, and γ chains [6]. It regulates cell adhesion, migration, differentiation, and proliferation, contributing to tissue architecture, homeostasis, local tumor invasion, and metastasis. Increased laminin expression has been observed in several epithelial malignancies, including lung, colorectal, prostate cancer and squamous cell carcinomas, among others, and is strongly associated with increased invasiveness and poor clinical outcomes [7].

Accumulating evidence indicates that laminin expression has prognostic relevance in breast cancer. Laminin expression has been associated with lymphovascular invasion, lymph node metastasis, and poorer survival. Immunohistochemistry (IHC) assessment of laminin may provide prognostic significance when combined with established clinicopathological parameters [8]. Therefore, evaluating laminin expression in breast carcinoma may provide valuable prognostic information and could potentially serve as a predictive biomarker for tumor progression or therapeutic response [9]. This study evaluated laminin expression in invasive breast carcinoma and its relationship with clinicopathological parameters.

2. MATERIALS AND METHODS

2.1 Study design and Duration

This cross-sectional study has been performed in the city of Basrah, Iraq, between February 1, 2025, and November 30, 2025, in Al-Sadr Teaching Hospital and private laboratories.

2.2 Patient Selection

A total of 53 consecutive eligible archival cases were included during the specified period using convenience sampling. All participants had undergone either a mastectomy or a wide local excision with axillary lymph node dissection at the Al-Sadr Teaching Hospital and associated private laboratories. Patients who received chemotherapy or radiotherapy before the surgical operation were also strictly excluded.

2.3 Clinicopathological Data Collection

For each included case, comprehensive clinicopathological data were noted. The studied variables were patient's age and gender, tumor diameter, histological type, grade of the tumor, lymphovascular invasion, perineural invasion and confirmed lymph node status.

2.4 Histopathological Examination

Formalin-fixed, paraffin-embedded tissue blocks were retrieved from the archives of the histopathology department. 3-5 µm thick sections were cut and stained using standard H&E. The pathological type (WHO classification), grade (Nottingham modification of the Bloom-Richardson grading system), and stage (according to AJCC guidelines) were assessed for these slides.

2.5 Immunohistochemistry Materials

We used a polyclonal rabbit primary anti-laminin antibody (Code number: Z0097; Dako, Denmark), which recognizes the laminin α1, β1, and γ1 chains (predominantly laminin-111/211), for all IHC investigations.

2.6 Immunohistochemical Staining Procedure

An indirect polymer-based HRP method was performed manually for laminin staining. In brief, after performing deparaffinization and rehydration, the 3–5 µm tissue sections underwent standard antigen retrieval and peroxidase blocking. Afterward, the tissue was incubated with primary antibodies overnight (for 12-16 hours) and then visualized with DAB substrate chromogen followed by hematoxylin staining. Appropriate positive controls (using internal vascular basement membranes) and negative controls (omission of primary antibody) were included in each staining run.

2.7 Evaluation of Laminin Expression

Two histopathologists independently reviewed and assessed the immunostained slides. Positive IHC was defined as focal or diffuse brown staining of the cytoplasm and/or membrane in any tumor cell. Laminin expression was assessed by using a semiquantitative four-tier standardized scoring system:

- 0, 1+: Absent or weak staining (light yellow).
- 2+: Intermediate or moderate staining (yellow/brown).
- 3+: Strong staining (brown).

For statistical analysis, cases were categorized binary (Positive vs. Negative) to maintain statistical power given the sample size. A definitive cut-off was established where any staining of ≥1+ was considered positive. Inter-observer agreement was substantial (Kappa = 0.85), and any minor discrepancies were resolved by consensus.

2.8 Statistical Analysis

SPSS statistical software (version 26; IBM Corp., Armonk, NY, USA) was used to conduct all statistical analyses. Qualitative categorical data were presented in the form of percentages and frequencies. Associations between laminin expression and clinicopathological variables were analyzed using Pearson's Chi-Square test or Fisher's exact test based on data distribution. A p-value ≤ 0.05 was considered statistically significant.

2.9 Ethical Consideration:

The research protocol was reviewed and approved by the Research Ethics Committee of the College of Medicine, University of Basrah, in addition to the local health authorities. All patients provided written informed consent before data and sample collection. Tissue samples and patient data were anonymized, treated confidentially, and maintained privately.

3. RESULTS AND DISCUSSION

3.1 Baseline Demographic and Clinical Characteristics:

The progression and rapid dissemination of breast carcinoma depend on extracellular matrix remodeling [4], and laminin is a key promoter for tumor invasion and metastasis [7, 8, 9]. A total of 53 patients with invasive breast carcinoma were included. The female-to-male ratio was 52:1 (98.1% vs 1.9%) among the 53 patients. Mean age was 51.2 ± 11.1 years (range 28–75); 62.3% of cases were over the age of 50 years. The tumor size in 41 cases (77.4%) was >2 cm (Table 1).

Table (1): Baseline Demographic and Clinical Characteristics

Variable		Frequency	Percent
Age (years)	**50 years or younger**	20	37.7
	Older than 50 years	33	62.3
Tumor size (cm)	**≤ 2 cm**	12	22.6
	>2 cm	41	77.4

3.2 Histopathological Characteristics

Out of the total cases, 50 cases (94.3%) were diagnosed as invasive ductal carcinoma (NST), while invasive lobular, micropapillary, and mucinous carcinoma subtypes each accounted for 1.9% of cases. Regarding tumor grade, 24 cases (45.3%) were grade 2 and 29 cases (54.7%) were grade 3. None of the cases demonstrated grade 1 morphology. Lymph node involvement was present in 29/53 cases (54.7%), while 24/53 cases (45.3%) were lymph node-negative. Lymphovascular invasion was identified in 29/53 cases (54.7%), while 24/53 cases (45.3%) showed no lymphovascular invasion. Perineural invasion was identified in 16/53 cases (30.2%) (Table 2).

Table (2): Baseline Histopathological Characteristics

Variable		Frequency	Percent
Histopathological Type	**Invasive ductal carcinoma (NST)**	50	94.3
	Invasive lobular carcinoma	1	1.9
	Invasive micropapillary carcinoma	1	1.9
	Mucinous carcinoma	1	1.9
Grade	**1**	0	0.0
	2	24	45.3
	3	29	54.7
Lymph Node Status	**Positive**	29	54.7
	Negative	24	45.3
Lymphovascular Invasion	**Identified**	29	54.7
	Not identified	24	45.3
Perineural Invasion	**Identified**	16	30.2
	Not identified	37	69.8

3.3 Laminin expression

Laminin expression was positive in 37 of 53 cases (69.8%), whereas 16/53 cases (30.2%) were negative for laminin immunostain (Fig 1).

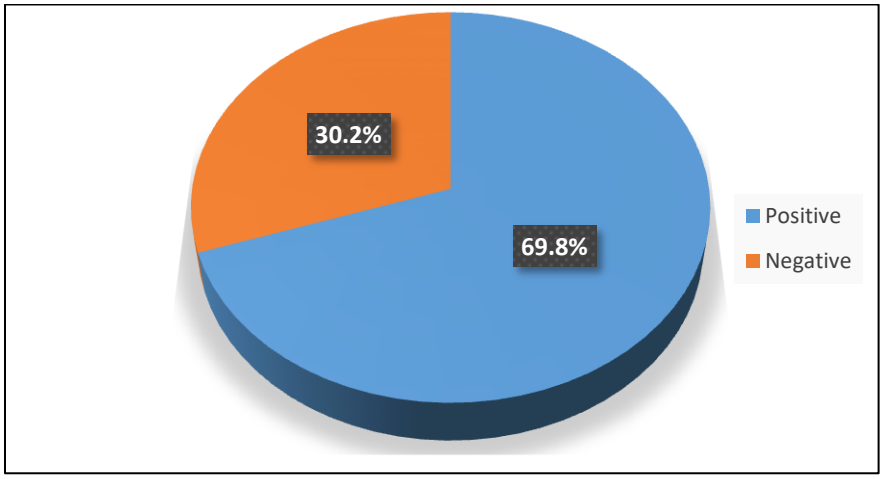

Fig (1): Distribution of laminin expression among the studied group

3.4 Association of Laminin Expression with Clinical Features

Thirty-three patients were older than 50 years; 28 cases (84.8%) were positive for laminin expression. In 20 patients aged 50 years or younger, only 9 cases (45.0%) were positive for laminin expression. There was a significant statistical association between laminin expression and older age (>50 years). Among tumors measuring >2 cm, 36 of 41 cases (87.8%) were laminin-positive, whereas only 1 of 12 cases (8.3%) with a tumor size ≤2 cm was laminin-positive. A statistically significant association was identified between laminin expression and larger tumor size (>2 cm) ($P < 0.0001$) (Table 3).

Table (3): Association of Laminin Expression with Patient Age and Tumor Size

Variables		Laminin		Total	P-value*
		Positive	Negative		
Age (years)	≤50 years	9 (45.0%)	11 (55.0%)	20 (100.0%)	0.002
	>50 years	28 (84.8%)	5 (15.2%)	33 (100.0%)	
Tumor size (cm)	≤ 2 cm	1 (8.3%)	11 (91.7%)	12 (100.0%)	<0.0001
	>2 cm	36 (87.8%)	5 (12.2%)	41 (100.0%)	

***Pearson Chi-Square test or Fisher's Exact Test.**

3.5 Association of Laminin Expression with Histopathological Features

Laminin expression was positive in 36/53 cases (72.0%) of IDC (NST). The mucinous carcinoma case showed positive laminin expression, whereas invasive micropapillary and invasive lobular carcinomas were negative. However, there was no statistically significant association between histopathological type and laminin expression (P=0.220). Laminin positivity was observed in 25 out of 29 cases (86.2%) of grade 3 tumors, compared to 12 out of 24 cases (50.0%) of grade 2 tumors. There was a significant statistical association between laminin expression and higher tumor grade (P=0.004). Twenty-four (82.8%) out of 29 cases with lymph node metastasis had positive laminin expression. Thirteen (54.2%) out of 24 cases without lymph node metastasis had positive expression of laminin. A significant statistical association (P=0.024) was found between the presence of laminin expression and lymph node involvement by cancer. A higher proportion of laminin-positive cases was detected among tumors with identified lymphovascular invasion (23 out of 29 cases; 79.3%). However, no significant statistical association (P=0.098) was found between the expression of laminin and lymphovascular invasion. Perineural invasion was identified in 16/53 cases; 12 of these cases (75.0%) exhibited positive laminin expression. In contrast, among the 37/53 cases without perineural invasion, laminin positivity was observed in 25 cases (67.6%). Moreover, no significant statistical association (P=0.588) was found between laminin expression and perineural invasion (Table 4).

Table (4): Association of Laminin Expression with Histopathological Features

Variables		Laminin		Total	P-value*
		Positive	Negative		
Histological type	Invasive ductal carcinoma (NST)	36 (72.0%)	14 (28.0%)	50 (100.0%)	0.220
	Invasive lobular carcinoma	0 (0.0%)	1 (100.0%)	1 (100.0%)	
	Invasive micropapillary carcinoma	0 (0.0%)	1 (100.0%)	1 (100.0%)	
	Mucinous carcinoma	1 (100.0%)	0 (0.0%)	1 (100.0%)	
Grade	2	12 (50.0%)	12 (50.0%)	24 (100.0%)	0.004
	3	25 (86.2%)	4 (13.8%)	29 (100.0%)	
Lymph node status	Positive	24 (82.8%)	5 (17.2%)	29 (100.0%)	0.024
	Negative	13 (54.2%)	11 (45.8%)	24 (100.0%)	
Lymphovascular Invasion	Identified	23 (79.3%)	6 (20.7%)	29 (100.0%)	0.098
	Not identified	14 (58.3%)	10 (41.7%)	24 (100.0%)	
Perineural Invasion	Identified	12 (75.0%)	4 (25.0%)	16 (100.0%)	0.588
	Not identified	25 (67.6%)	12 (32.4%)	37 (100.0%)	

***Pearson Chi-Square test or Fisher's Exact Test.**

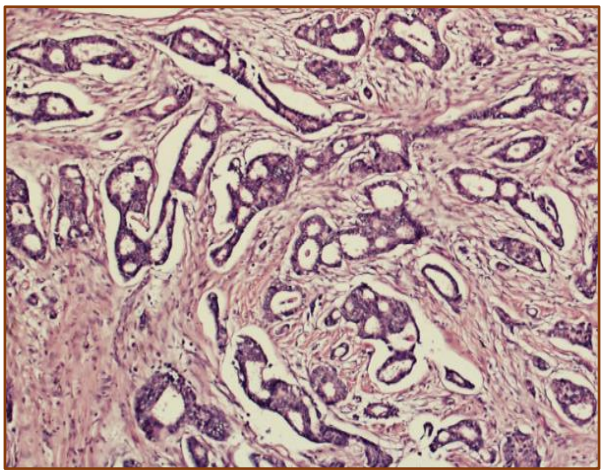

Fig (2): Invasive ductal carcinoma (NST), grade 2. H&E stain (100X)

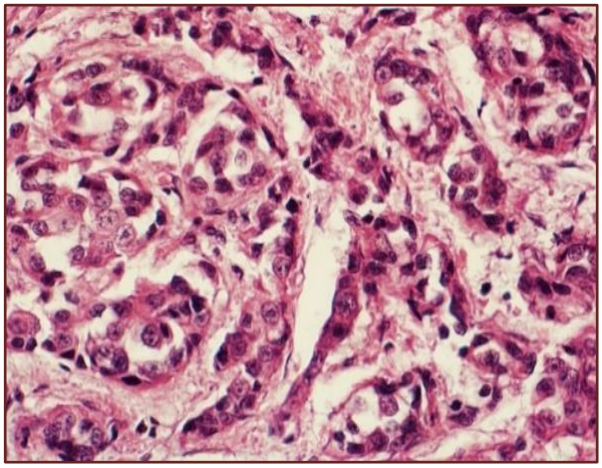

Fig (3): Invasive ductal carcinoma (NST), grade 3. H&E stain (400X)

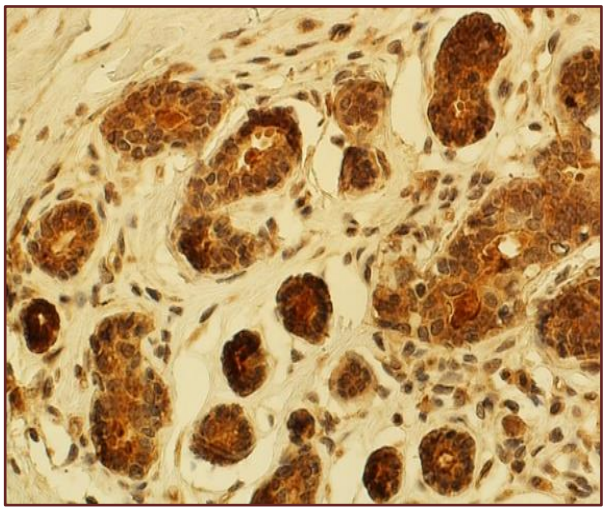

Fig (4): Strong (3+) membranous and cytoplasmic laminin staining in invasive ductal carcinoma (NST) IHC (400X)

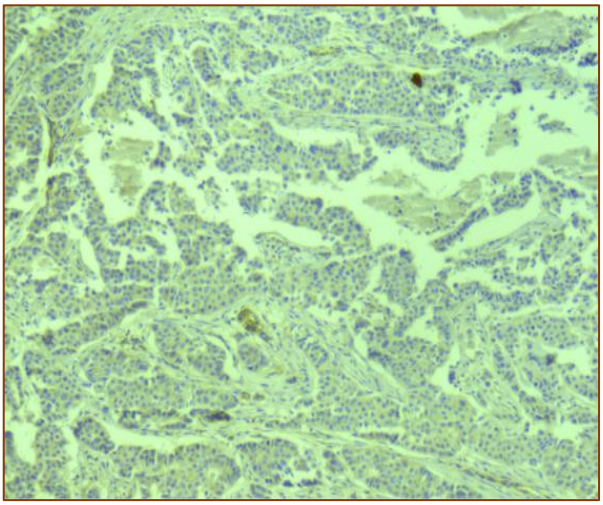

Fig (5): Negative laminin expression in invasive ductal carcinoma, IHC (100X)

3.6 Baseline Demographic and Clinical Characteristics

In this study, the mean age of patients was 51.2 ± 11.1 years. This is in line with several recent Iraqi studies. For instance, a study from Basrah documented a mean age of 50 years [10], and another multi-center Iraqi study reported a mean age of 51.2 years [11], as well as a study from Saudi Arabia that reported a mean age of 49 years [12]. However, some other Iraqi studies have shown slightly lower mean ages, such as 48 years and 43.5 years [13] [14].

3.7 Histopathological Characteristics

The predominant histological type in this study is invasive ductal carcinoma (NST) (94.3%). This result is consistent with other studies carried out in Basrah (85.5%) [10], Middle Euphrates (91.8%) [15], and Saudi Arabia (78.0%) [12]. Regarding tumor grading, grade 3 breast cancer (54.7%) was higher than grade 2 (45.3%) in the current study. This finding contrasts with most Iraqi studies that reported grade 2 as the most common grade [10,15, 16]. The difference may be due to the small sample size, the inclusion of predominantly advanced cases and biologically aggressive subtypes, and delayed clinical presentation; minor interobserver and technical variations in grading may also contribute.

3.8 Laminin expression

In the present study, laminin expression was observed in approximately two-thirds of the cases (69.8%). This result was in line with a study conducted in Egypt, which reported a laminin expression rate of 67.5% [17], as well as other international studies that have shown expression rates of 73.2% [18] and 70.0% [19]. Conversely, an Indian study reported a lower laminin expression rate of 53.5% [20]. The observed variations in laminin expression across different populations could be attributed to genetic and epigenetic differences among diverse ethnic groups, varying environmental exposures, and technical disparities in immunohistochemistry protocols (e.g., scoring criteria). While this study evaluated overall laminin expression using a polyclonal antibody targeting classic chains α1, β1, γ1), specific isoforms such as laminin-332 have also been implicated in breast cancer invasion, highlighting a potential area for future isoform-specific studies.

3.9 Association of Laminin Expression with Clinical Features

In this study, a significant association was observed between laminin expression and older age (>50 years). This finding was in agreement with studies from India [20, 21]. In contrast, in Nigeria [22] and in the USA [18], found a significant correlation between laminin expression and younger patients (<50 years). Furthermore, there was a significant association between laminin expression and tumor size, where a higher laminin expression (87.8%) was noted in larger tumors (>2 cm), which is consistent with a study reporting an 86.7% expression rate [20]. However,

other studies did not show any significant association between laminin expression and tumor size [22, 23], which could be attributed to variations in sample sizes, diverse population demographics, or differences in scoring criteria.

3.10 Association of Laminin Expression with Histopathological Features

There was no statistically significant association between histological type and laminin expression in this study. This is in accordance with other international studies [20-22] However, laminin expression showed a statistically significant association with histological grade, being more frequent in grade 3 tumors (86.2%), which was in agreement with study with 52.24% expression rate [18], unlike other studies that did not demonstrate a statistically significant correlation with tumor grade [20-23].

A significant association was found between lymph node involvement and laminin expression in this study, with an expression rate of 82.8%; this was in agreement with a study (83.3%) [20]. While other studies found no association between laminin expression and lymph node involvement [17, 22]. Some studies found a significant association between laminin expression and lymphovascular invasion [20, 21]. However, the opposite was observed in the current study and recorded by a Nigerian study, in which there was no significant association between laminin and lymphovascular invasion [22]. However, a noticeable clinical trend toward higher laminin positivity was observed in LVI-positive cases (79.3% vs. 58.3%), which might achieve statistical significance in larger study cohorts. To date, there is a paucity of breast cancer-specific studies investigating the association between laminin expression and perineural invasion. In the current study, although a higher laminin positivity rate was observed in the PNI-positive group (75.0% vs. 67.6%), this difference did not reach statistical significance, which is in agreement with the findings of other studies [21]. This lack of statistical significance is likely due to the analysis being underpowered for this specific variable, given the small number of PNI-positive cases (n=16). In BC patients and RT workers, high XRCC1 levels were linked to RT parameters, suggesting that XRCC1 may predict RT responsiveness and DNA repair efficacy [24].

A limitation of the current study is its reliance on univariate statistical analysis due to a small sample size (n=53), which made robust multivariate logistic regression unfeasible. This increases the risk of Type I error, so findings should be interpreted cautiously. Additionally, data on key breast cancer molecular markers, such as hormone receptor status, HER2 status, and the Ki-67 index, were unavailable due to the retrospective design and archival constraints. Future studies should include these parameters to better evaluate laminin's independent value alongside established prognostic markers.

4- CONCLUSION

The study cohort consisted almost entirely of female patients, with the vast majority presenting with high-grade invasive ductal carcinoma. Laminin was found to be notably overexpressed in most of the evaluated cases. Importantly, a strong positive correlation was found between high expression of laminin and adverse clinicopathological features such as the patients' age, pathological grade, diameter of tumor development, and positive lymph nodes. In contrast, laminin positivity was not significantly associated with the individual histopathologic type and lymphovascular or perineural invasion status.

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