

## Immunohistochemical evaluation of laminin expression in breast cancer among Sudanese women in Khartoum state, Sudan 2017

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### Abstract:

**Background:** *Cancers are heterogeneous tissues comprised of multiple components, including tumor cells and microenvironment cells. Laminin is a glycoprotein with diverse functions in carcinogenesis including cell proliferation, invasion, metastases and epithelial-mesenchymal transition (EMT). In breast cancer (BC) laminin expression is speculated to be associated with unfavorable clinicopathological and molecular characteristics.*

**Method:** *This descriptive, cross sectional study was performed to Evaluate laminin expression in breast cancer among Sudanese women, in This Cross sectional study which conducted at Radiation isotopes center of Khartoum (RICK) in Khartoum (Sudan) from December 2016 to April 2017. we aimed to evaluate the immunohistochemical expression of laminin tumor marker in breast cancer patients. Forty Samples of formalin fixed wax embedded blocks were selected and sectioned and immunohistochemically stained to evaluate laminin expression.*

**Results:** *Out of 40 patients with Breast cancer, most participants were in age group between 40-50 years, four cases (22%) at age 40-50 were positive for laminin expression, while fourteen (78%) were negative & according to the distribution of receptor status five cases (27.5%), four cases (22%) of triple negative TN and not triple negative were positive respectively. most of positive expression were associated with Grade I while most of negative with Grade II.*

**Conclusions:** *The laminin expression results were insignificantly correlated with Age, grade, diagnosis and receptor status, further studies including large number of participants required.*

**Key words:** Breast cancer, laminin, Sudan, Immunohistochemistry

## INTRODUCTION

Breast cancer refers to cancers originating from breast tissue, most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk. Breast cancer is the most frequently diagnosed cancer in women, with an estimated 1.38 million new cases per year (<http://www.merck.com/mmhe/sec22/ch251/ch251f.html>). Fifty thousand cases in women and 400 in men are recorded each year in the UK alone. Breast cancer is the most common cause of cancer death in women and the second most common cause of cancer death in women in the U.S. There are 58,000 deaths per year from breast cancer worldwide making it the most common cause of female cancer death in both the developed and developing world [1] Cancer cells are very similar to cells of the organ from which they originated and have similar (but not identical) DNA and RNA. This is the reason why they are not very often detected by the immune system, in particular, if it is weakened [2].

In Sudan, breast cancer accounts for around a third of all cancers. previous studies showed that the prevalence of

advanced, stage III or worse metastatic disease was higher in women living in rural areas than it was in women living in urban areas in Sudan [3].

Laminins are cell adhesion molecules that comprise a family of glycoproteins found predominantly in basement membranes, which are the thin sheets of extracellular matrix that underline epithelial and endothelial cells and surround muscle cells, Schwann cells, and fat cells. Many laminins self-assemble to form networks that remain in close association with cells through interactions with cell surface receptors [4]. Laminins are vital for many physiological functions. They are essential for early embryonic development and organogenesis and have crucial functions in several tissues including muscle, nerve, skin, kidney, lung, and the vasculature [5].and it's known to stimulate the migration of various cells including carcinoma cells [4]. Laminins are heterotrimers containing  $\alpha$ ,  $\beta$  and  $\gamma$  chains forming a cross-shaped structure [5].The major functions of LN332 include binding of epithelial cells to the basement membrane through the formation of hemidesmosomes, and the migration of epithelial cells during wound repair [6].

The main role of laminin332 LN332 in normal tissues is in the maintenance of epithelial-mesenchymal cohesion in tissues exposed to external disruptive forces [7, 8]. LN332 seems to have a more complex role in cell migration and tumor invasion [6]. Since these properties are required for metastasis [9], LN332 has been implicated in tumor progression [10].

The role of LN332 in breast carcinoma is not clear. Although the earliest studies reported that the LN332 at the invasive edge of breast carcinomas [8], other investigations concluded that LN332 expression was lost as breast carcinoma progressed[11].

## **MATERIALS AND METHODS:**

In this Cross sectional study which conducted at Radiation isotopes center of Khartoum (RICK) in Khartoum (Sudan) from December 2016 to April 2017. we aimed to evaluate the immunohistochemical expression of laminin tumor marker in breast cancer patients. Forty Samples of formalin fixed wax embedded blocks were selected and sectioned and immunohistochemically stained to evaluate laminin expression. All of them were previously diagnosed and pathologically confirmed with breast cancer.

### **Methods**

The method used to evaluate the breast cancer was laminini mmunohistochemical marker. Three micrometers thickness sections from each paraffin block were cut by rotary microtome in positive charge (Fisher brand) Immunohistochemistry slides. The sections were hot dried in oven at 60°C over night following deparaffinization in xylene, slides were rehydrated through a graded series of alcohol and placed in running water. hematoxylin and eosin slides were made of each sample for histological evaluation.

Samples steamed for antigen retrieval for laminin using Pre Treatment system from Dako PT link as follow:

Briefly, slides placed in slide tank containing enough sodium citrate buffers (pH 9.0) to cover the sections, then boiled at high Temperature (99°C) for 20 minutes then sections allowed to cool at RT.

Endogenous peroxides activity blocked with 3% hydrogen peroxides in methanol for 10 minutes, then slides incubated with 100µl of primary monoclonal antibody laminin for 20 minutes at room temperature in a moisture chamber, and then rinsed in Phosphate buffer saline after washing with PBS

for 3 minutes, binding of antibodies detected by incubating for 20 minutes with dextran labeled polymer (Thermo kit).

Finally, the sections washed in three changes of PBS, followed by adding 3,3 diaminobenzidine tetra hydrochloride (DAB) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 min. Then slides counterstained with haematoxylin, slides dipped for one minute and then blued using amonical tap water for two seconds. For each run of staining, positive and negative control slides also prepared. The positive control slides contained the antigen under investigation and the negative control slides prepared from the same tissue block but incubated with PBS instead of the primary antibody. Each slide evaluated with investigator and each slides interpreted negative or positive for laminin marker. Positive laminin staining identified in form of dark nuclear staining obtained results and variables arranged in standard master sheet, and then entered a computer program SPSS and analyzed.

### **Statistical analysis**

The results of the study were statistically analyzed using SPSS version 21 statistical program. Data were expressed as mean $\pm$ SD for quantitative variables, numbers and percentage. For categorical variables, student t test was used. For statistical analysis of Gleason's grading Spearman's statistical test was used.  $P < 0.05$  was considered the significant limit.

## **RESULTS**

The study included patients diagnosed with breast cancer to evaluate laminin expression. A total of forty participants that enrolled for the study were within the study period. Most of the study participants were within the age group 40-50 years, followed by group 30-40 years, while age group less than 30

years constituted the least group (**Table 2**) . In (**Table 2**) show there 4 (22%) positive of laminin expression at age 40-50 while 14 (78%) were negative(p.value 0.344). also when show distribution of receptor status the number of positive were 5 in triple negative TN and 4 in not triple negative NTN of a total of participants(p.value0.06). p.value(0.09) of laminin expression among breast cancer was that consider insignificant according to grade of disease and diagnosis level showed it in (**Table 4**)

**Table (1) show the distribution of the study sample according to age group**

Age in years	n	%
Less than 30	1	2.5%
30-40	11	27.5%
<b>40-50</b>	18	45%
50-60	8	20%
More than 60	2	5%
<b>Total</b>	40	100%

**Table (2) show the Distribution of the Laminin expression according to age group**

Age	Positive
Less than 30	1(0%)
30-40	2(18%)
<b>40-50</b>	4(22%)
50-60	2(25%)
More than 60	1(50%)

**P.value :0. 344**

**Table (3) show the Distribution of the Laminin expression according to receptor status**

Receptor status	Laminin expression	
	Positive	Negative
Triple negative	5 (38%)	8 (62%)
Non triple negative	4(15%)	23(85%)

**P.value : 0.09**

**Table (4) show the Distribution of the Laminin expression according to histological grade**

Grade	Laminin expression	
	Positive	Negative
Grade I	4(44%)	5(56%)
Grade II	0(0%)	10(100%)
grade III	1(11%)	8(89%)
Non grade	4(33%)	8(67%)

**P.value :0.06**

## **DISCUSSION**

Laminin expression has been associated with malignant transformation as well as worse clinical outcome in breast cancer & it is used in diagnosis, therapy planning & sub typing for breast cancer.

In these study we examined the expression of Laminin among Sudanese patients using of immunohistochemical method , we found that the results are compatible with other international studies.

In our study we found that 13 samples which represent 32.5% of examined samples have been showed positive result reflecting the lower significant relationship between Laminin and breast carcinoma in Sudanese patients.(table1).

In details total of 18 patients which represent 45% grouped in between (40 –50) years old , 4 positive (22%) where 14 negative (78%).(tabel1).Followed by 11 patients which represent 27.5% grouped in between (30 – 40) years old , 1 positive (9%), where 10 negative (91%).(table1)(table2).No significant correlation to age group (p.v 0.344).

In triple negative and non triple negative 9 samples (22.5%) have been showed positive Laminin expression where 31 samples(77.5%) showed negative result reflecting that there is no correlation between Laminin expression and receptors distribution (P.value 0.09), (table3).

Laminin expression in breast cancer patients was insignificantly correlated (P.value 0.16), further more studies should be carried for more evaluation of Laminin immnohistochemical expression among breast cancer patients in Sudan.

## **CONCLUSION**

The laminin expression results were insignificantly correlated with Age, grade, diagnosis and receptor status, further studies including large number of participants required for more evaluation of Laminin expression.

## **RECOMMENDATION**

We recommend designing cohort study for Breast cancer to evaluate the expression of laminin also we recommend increasing sample size.

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