Claudin-4 Expression in Triple Negative Breast Cancer: Correlation with Androgen Receptors and Ki-67 Expression

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Introduction

- Breast cancer is the most common malignancy in women and the leading cause of cancer mortality worldwide, being responsible for more than 458, 000 deaths annually.
- In Egypt, breast cancer is the most common cancer among women, representing 32% among Egypt National Cancer Institute series in the year 2014.

- Triple-negative breast cancer(TNBC), immune-histochemically, defined by lack of expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2(Her2), is an important phenotype of breast cancer that accounts for approximately 10–15% of all breast cancers.

- Although TNBC accounts for a relatively small number of breast cancer cases, but still represent a focus of increasing interest at the clinical, biological and epidemiological level, due to the aggressive behavior of the tumor, poor prognosis, fewer treatment options and higher death rate than other subtypes of breast cancers.

- The proliferation marker Ki-67 has repeatedly been confirmed as an independent predictive and prognostic factor in early breast cancer. Breast cancer with high Ki-67 expression responds better to chemotherapy, but is associated with poor prognosis.

- Claudins are the major component of the tight junction, and 24 members of this family of proteins have been identified to date. They are small proteins ranging in size from 22 to 27 kD and are encoded by at least 17 human genes located on 12 different chromosomes.
- This wide distribution of the claudin genes among many different chromosomes may reflect the multifunctional characteristics of these proteins.

- Several members of the claudin family have been found to be overexpressed in a wide variety of cancers.
- This includes claudin-4 in gastric cancer and pancreatic cancer; and claudins-3 and -4 in prostate cancer, ovarian cancer, endometrial cancer and breast cancer.
- To date, only a few studies have addressed the role of claudins in breast cancer, especially triple negative breast cancer, and findings on their function remain controversial.

- Androgens, including testosterone and dihydrotestosterone, function by binding to and activating intracellular Androgen receptors (ARs).
- AR like estrogen receptor (ER) and progesterone receptor (PR), belongs to steroid nuclear receptor family.
- AR as a member of the nuclear receptor superfamily is known to be involved in a complex network of signaling pathways that collectively regulate cell proliferation.
- Its expression is abundant in normal breast epithelial cells.
- AR has been found to be an important target in prostate cancer, and it has recently been considered as a potential biomarker in breast cancer.

- It is commonly expressed in ductal carcinoma in situ and invasive breast carcinoma.
- Moreover, AR can be co-expressed with ER and PR.
- Roles of AR in breast cancer development and progression have not been very clearly understood.
- Some researchers have reported that the expression of AR is associated with a better prognosis**.** But the prognostic significance varies with the different molecular subtypes of breast cancer.

- Recent studies suggest a possible anti-proliferative effect of AR stimulation and pathway activation in breast cancer.
- Also claudin-4 aberrant expression has been associated with structural and functional damage of tight junctions in several cancers, including breast cancer.
- However, little is known about the correlation status between claudin-4, AR and ki-67 expression in TNBC.

Aim of The Work

In this study, we aimed to:

- Identify the clinicopathological associations and prognostic value of claudin-4 expression in triple negative breast cancer and
- Correlate claudin-4 expression with AR status and Ki-67expression.

Material and Methods

- The selected cases comprised 56 cases of triple-negative primary invasive ductal breast carcinoma, based on the ER, PR and the Her-2 negativities.
- The sections were immunostained for:

1-Claudin-4

2-AR

3-Ki-67.

Evaluation of immunohistochemical results:

Claudin immuno-reactivity:

- was evaluated semi-quantitatively in tumor cells demonstrating distinct cytoplasmic reaction in at least five high-power fields at x400 magnification
- Based on combined score of the intensity (0, no stain; 1, weak; 2, moderate; 3, strong) and the percentage of stained tumor cells (0=<5%;1=5-25%; 2= 26–50%; and 3= >51%).

Claudin immuno-reactivity:

- The two scores were multiplied to give an overall score of 0–9, of which 0 was considered negative,1–2 was considered weak, 3–6 moderate, and 9 strong staining.
- Negative and weak expression was considered as low claudin expression, whereas moderate and strong as high claudin expression.

- ***Evaluation of AR***: the tumor was considered to be positive for AR if the percentage of cells showing positive nuclear staining was more than or equal to 10%.
- ***Ki-67 evaluation:*** Cells showing nuclear staining for Ki-67 were counted and expressed as a percentage. High expression of Ki-67 was defined as ≥10%, because 10% as cutoff provided the best prognosis-prediction results in several studies .

Results

Patient characteristics:

- Most of the patients (82.14%) were older than or equal to 50 years.
- Most carcinomas were of intermediate (46.43%) and high grades (44.64%),
- The majority of cases (89.3%) are associated with positive nodal involvement.
- Most tumors (50%) were between 2 and 5 cm in diameter, and 37.5 % were larger.

Clinico-pathological variables	N=56	%
Age (years)		
Premenopausal < 50 years	10	17.86
Postmeopausal ≥50 years	46	82.14
Tumor size		
T1 (<2cm)	7	12.5
T2 (2-5cm)	28	50
T3 (>5cm)	21	37.5
Tumor grade		
I	5	8.9
II	26	46.43
III	25	44.64
Nodal status		
N0	6	10.7
N1	10	17.86
N2	21	37.5
N3	19	33.93
Distant metastasis		
M0	38	67.86
M1	18	32.14

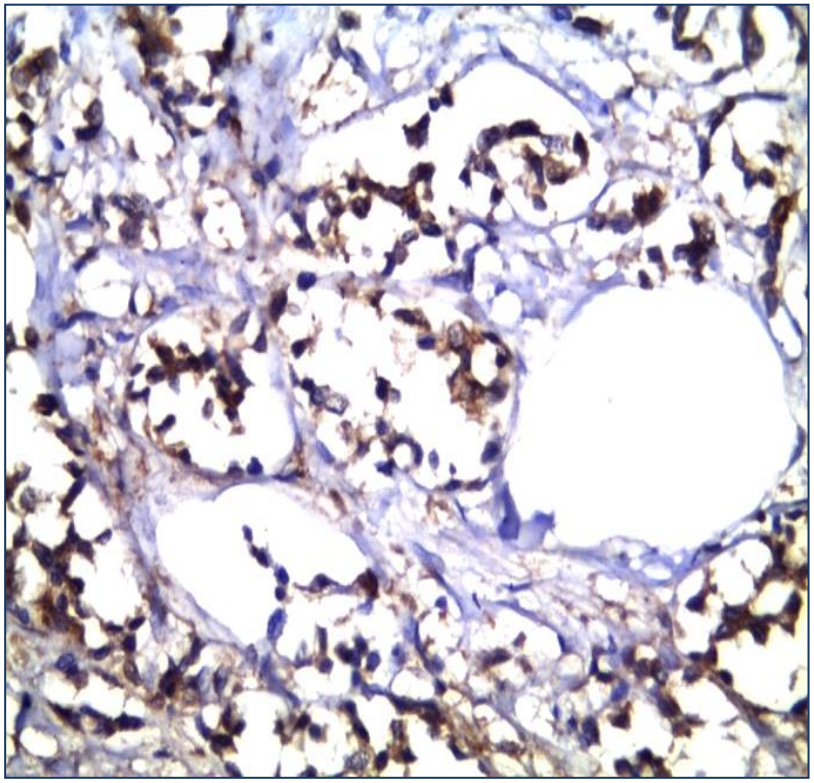
Expression of claudin-4:

- High levels of claudin-4 cytoplasmic expression were detected in 66.1% of TNBC cases.
- This high expression was significantly associated with higher age at diagnosis, large tumor size, high grade, advanced nodal involvement and metastasizing tumors (***p*<0.05**).
- There was a positive correlation with age, tumor size, grade, nodal involvement and distant metastasis**.**

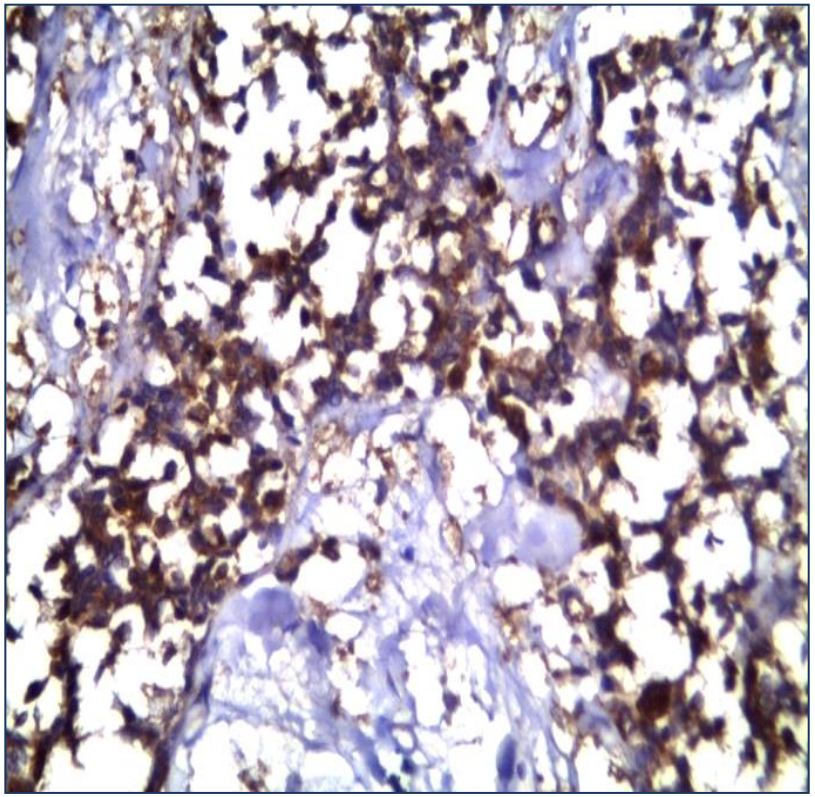
		Claudin score				Chi Square	p. value
		Low expression ≤2		High Expression >2			
Age	Range	44-61		48-77		9.635	<0.05*
	Mean±SD	53.15+6.06		62.55+7.12			
		N	%	N	%		
Tumor size	T1	7	35%	0	0%	15.296	<0.05*
	T2	9	45%	19	52.78%		
	T3	4	20%	17	47.22%		
	Total	20	100%	36	100%		
Grade	I	5	25%	0	0%	28.359	<0.05*
	II	15	75%	11	30.6%		
	III	0	0%	25	69.4%		
	Total	20	100%	36	100%		
Nodal status	N0	6	30%	0	0%	35 .487	<0.05*
	N1	9	45%	1	2.8%		
	N2	5	25%	16	44.4%		
	N3	0	0%	19	52.8%		
	Total	20	1005	36	100%		
Metastasis	M0	20	100%	18	50%	14.737	<0.05*
	M1	0	0%	18	50%		
	Total	20	100%	36	100%		

Claudin-4

low expression of claudin-4 in a case of TNBC *(IHCx400)*.



High expression of claudin-4 in a case of TNBC *(IHC x400).*



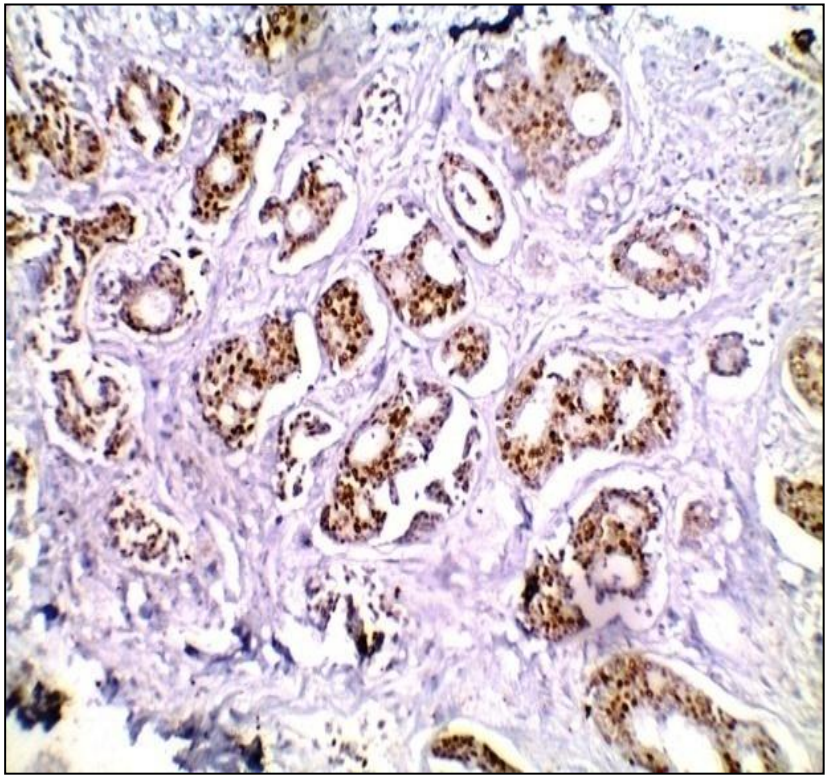
AR immuno-staining:

- Positive AR immuno-staining was detected in 29 cases (51.78%).
- A negative correlation was found between AR expression and different clinicopathological factors.
- AR was significantly expressed in patients with younger age at diagnosis, smaller tumor size, lower histologic grade, no or minimal nodal involvement and non-metastasizing tumors (***p*<0.05**).

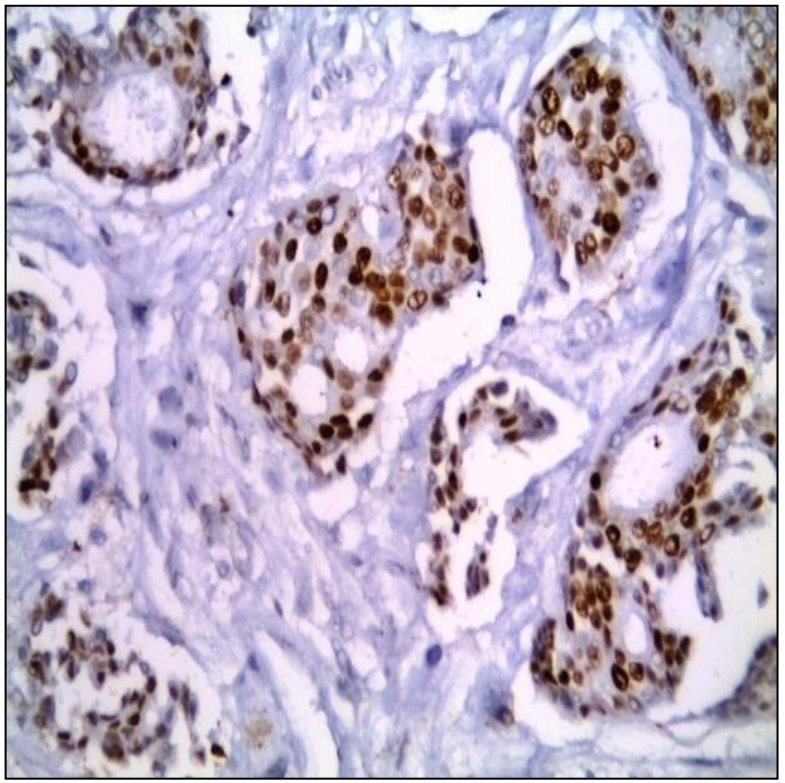
		AR				Chi Square	p. value
		-ve		+ve			
Age	Range	52-77		44-61		6.959	<0.05*
	Mean±SD	65.29±5.36		53.51±5.74			
		N	%	N	%		
Tumor size	T1	0	0%	7	24.14%	31.159	<0.05*
	T2	7	25.93%	21	72.41%		
	T3	20	74.07%	1	3.45%		
	Total	27	100%	29	100%		
Grade	I	0	0%	5	17.2%	48.606	<0.05*
	II	2	7.4%	24	82.8%		
	III	25	92.6%	0	0%		
	Total	27	100%	29	100%		
Nodal status	N0	0	0%	6	20.7%	33.705	<0.05*
	N1	1	3.7%	9	31%		
	N2	7	25.9%	14	48.3%		
	N3	19	70.4%	0	0%		
	Total	27	100%	29	100%		
Metastasis	M0	9	33.3%	29	100%	28.491	0.001*
	M1	18	66.7%	0	0%		
	Total	27	100%	29	100%		

AR

AR nuclear positivity in TNBC *(IHCx200)*.



Higher magnification of the previous case *(IHCX400)*.



Comparison of claudin-4 expression in AR positive and AR negative TNBC:

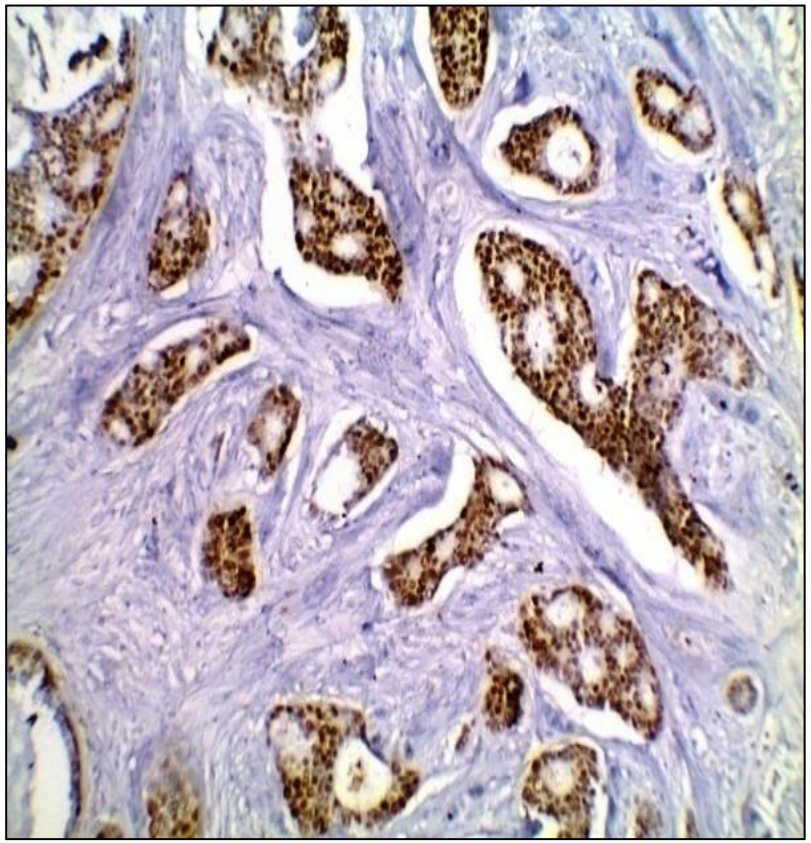
- We compared claudin-4 expression between AR positive and AR negative TNBC cases.
- There was a negative correlation with AR expression (r=-0.719; p=0.001).
- As claudin-4 expression in AR negative tumors was significantly higher than in AR positive tumors (p=0.001).

Ki-67 Expression in TNBC

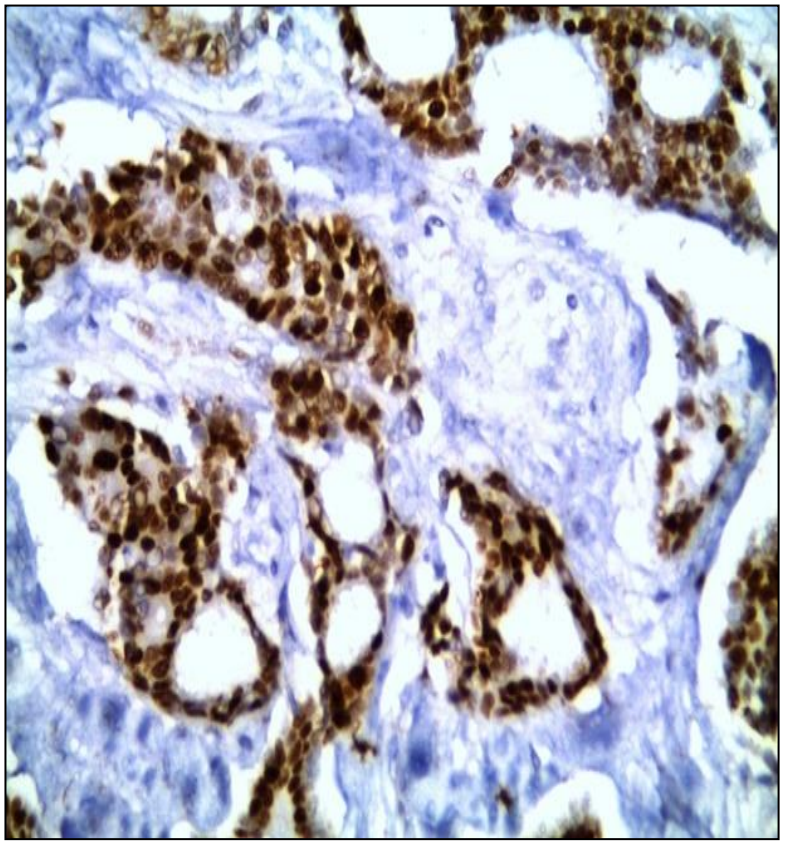
		Ki67				Chi Square	p. value
		-ve		+ve			
Age	Range	44-61		60-77		6.326	0.009*
	Mean±SD	53.61±5.61		66.12±4.56			
		N	%	N	%		
Tumor size	T1	7	22.58%	0	0%	24.351	<0.05*
	T2	21	67.74%	7	28%		
	T3	3	9.68%	18	72%		
	Total	31	100%	25	100%		
Grade	I	5	16.1%	0	0%	41.084	0.001*
	II	24	77.4%	2	8%		
	III	2	6.5%	23	92%		
	Total	31	100%	25	100%		
Nodal status	N0	6	19.4%	0	0%	36.943	0.001*
	N1	9	29%	1	4%		
	N2	16	51.6%	5	20%		
	N3	0	0%	19	76%		
	Total	31	100%	25	100%		
Metastasis	M0	31	100%	7	28%	32.893	0.004*
	M1	0	0%	18	72%		
	Total	31	100%	25	100%		
AR	-ve	2	6.5%	25	100%	48.507	0.001*
	+ve	29	93.5%	0	0%		
	Total	31	100%	25	100%		
Claudin score	≤2	20	64.5%	0	0%	25.090	0.001*
	>2	11	35.5%	25	100%		
	Total	31	100%	25	100%		

KI-67

Ki-67 nuclear positivity in TNBC *(IHCx200)*.



Higher magnification of the previous case *(IHCx400).*



Comparison of claudin-4 expression between Ki-67 positive and Ki-67 negative TNBC:

- Claudin-4 expression was positively correlated with Ki-67 expression (r=0.669; *p*=0.002).
- All TNBC cases with high Ki-67 expression level showed significantly higher claudin-4 expression compared to 31 tumors with low ki-67 expression in which only 11 cases showed high claudin-4 expression (*p*= 0.001).

Relation between Ki-67 expression and AR status

- Positive Ki-67 immunostaining was detected in 25 cases of TNBC (44.64%), none of these cases showed AR expression, while among 31 cases (55.36%) of negative Ki-67 immunostaining, 29 of them were positive for AR.
- AR expression levels showed a significant negative correlation with Ki-67 expression (r = -0.931; p= 0.001) as Ki-67 expression among the AR-positive cases was significantly lower than that of the AR-negative cases (p=0.001).

	AR		ki67		Claudin-4 score	
	r.	p. value	r.	p. value	r.	p. value
Age	-0.733	0.001*	0.774	0.002*	0.561	0.003
Tumor size	-0.716	0.001*	0.638	0.001*	0.451	0.001*
Grade	-0.859	0.001*	0.791	0.002*	0.708	0.001*
Nodal status	-0.715	0.001*	0.715	0.001*	0.765	0.001*
Metastasis	-0.713	0.004*	0.766	0.001*	0.513	0.002*
AR			-0.931	0.001*	-0.719	0.001*
Claudin-4	-0.719	0.001*	0.669	0.002*		

Conclusion

- Our results support the concept that TN breast cancers are a heterogeneous group of tumors with different biological characteristics and clinical behavior.
- Thus high claudin-4 expression, negative AR expression and high Ki-67 index, would provide a strong prognostic power to differentiate the patients with worse outcome among TN breast cancer patients.
- Moreover targeted treatment for TN breast cancer cells expressing claudin-4 and pharmacological inhibition of AR which decreases the cell viability and tumor growth in TN breast cancers highly enriched for AR would be valuable for future therapies.

THANK YOU