

Immunohistochemical Characterization and Subclassification of Triple Negative Breast Cancer of Egyptian Female Patients

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Abstract

Background: Triple negative breast cancer is representing a heterogeneous group of breast cancer. Many trials were carried out for its subclassification by different markers. This classification also could help in new treatment modalities.

Aim of Study: To investigate and identify additional possible prognostic markers that can help in subclassification of triple negative breast cancer.

Patients and Methods: This retrospective study included 59 women with triple negative breast cancer referred to NCI, Cairo University, Egypt. Clinical data were collected. Immunohistochemical analyses were performed for the assessment of CK5/6, Epithelial growth factor receptor (EGFR), and Claudin 4. All cases were followed-up with a median follow-up time 40.5 months (range: 8-67).

Results: Among the studied cases (71.2%) were positive to CK5/6, (45.8%) were positive to EGFR, (89.8%) represented Claudin4 high. Expression of CK5/6 revealed a significant relationship with tumor size. Expression of EGFR revealed a significant relationship with stage of the tumor. Most of cases of Claudin 4 high represented high grade, high stage and node positivity, but not reached the significant level. There is significant relationship found between grade and DFS, as well as OS with grade, stage, occurrence of metastasis and node positivity.

Conclusion: Triple negative breast cancer is a heterogeneous molecular type of breast cancer. Basal markers such as CK5/6 and EGFR are used for identification of basal subgroup of TNBC. This subclassification of triple negative breast cancer helps in categorization of patients who are candidate for targeted therapy, such as tyrosine kinase inhibitors (TKIs) in cases positive for EGFR. Expression of Claudin 4 also helps in classification of triple negative breast cancer, and selection of cases that could be targeted by monoclonal antibodies.

Key Words: TNBC – EGFR – CK5/6 – Claudin 4.

Introduction

IN USA by the end of 2017, an estimated 252,710 new cases of invasive breast cancer were diagnosed

among women. Approximately 40,610 women died from breast cancer in 2017 [1].

In the National Cancer Institute, Cairo University (NCI-CU), breast cancer came as number one in ranking malignant tumors constituting 19.3% of total primary malignancies [2].

Previously the breast cancer classification was based on histological type as well as clinical and pathological parameters of the tumor (tumor size, grade, stage, and lymph node status) which was affecting treatment protocol [3]. The advances in breast cancer classification into molecular types represented a great discovery with marvelous effect on treatment strategy and prediction of prognosis [4].

The triple-negative breast cancer (TNBC) subtype accounts for approximately 15% of all breast cancers, and is defined pathologically by the absence of expression of the estrogen receptor (ER) and progesterone receptor (PR) and lack of over expression or amplification of the HER2/neu oncogene [5,6,7].

According to (NCI-CU) cancer 12-years registry; TNBC constitutes about 13.5% of breast cancer [2]. Triple negative breast cancer is the most serious group molecular subtypes of breast cancer as it carries bad prognosis, early recurrence and metastasis [8].

As chemotherapy is currently the only treatment option for patients with triple negative breast cancer. Attempts for investigating the expression of various immunohistochemical markers which could aid in further subclassification of TNBC and providing new insights for treatment options are on the run [9,10].

Such studies are actually lacking among Egyptian patients due to queries about cost effectiveness of such studies, their impact on the treatment

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options, and the availability of the targeted therapy against these antibodies.

Basal markers such as CK5/6 and EGFR are considered important in subclassification of the triple negative group and can also affect treatment options.

CK5/6 is a high molecular weight cytokeratin that is frequently applied in the panel of markers used to differentiate benign and malignant lesions of the breast [11] it identifies basal like phenotype in up to 81% of invasive breast carcinomas by using immunohistochemistry [12].

HER1/EGFR is a receptor tyrosine kinase (RTK) that belongs to the ErbB family, and a transmembrane protein comprising an extracellular ligand binding domain, transmembrane domain, and cytoplasmic tyrosine kinase domain and plays an important role in cell proliferation, migration and protection against apoptosis. HER1 protein could be targeted by monoclonal antibodies and/or tyrosine kinase inhibitors (TKIs). Given signal pathway complexity and HER family member cooperation, it may be better to simultaneously target a number of these receptors [9].

Claudin 4 is one of the transmembrane proteins which is important in tight junction of cells underwent many researches with great expectation to be targeted by targeting antibodies which used in designing new treatment planes [10].

The current study aimed to study the immunohistochemical expression of basal markers including CK5/6 and EGFR as well as Claudin 4 among triple negative breast cancer cases.

Patients and Methods

This retrospective study was carried out at the National Cancer Institute (NCI), Cairo University, Egypt, during the period from 2011 to 2014, diagnosed at pathology department. The study was approved by the Institutional Review Board of NCI. Written informed consent was obtained from all participants. The study included 59 women with triple negative breast cancer, which represented all case that met the inclusion criteria which were cases with available blocks, available follow-up with minimum time of two years. We excluded cases with lost files, unavailable blocks, incomplete follow-up data, and small non representative tumor samples.

Data of studied cases were collected from medical records in the pathology department as well as surgical oncology, medical oncology and biostatistics department. These included personal data,

clinical data, and pathologic data. All studied cases received the same anthracyclin based chemotherapy.

Hematoxylin and Eosin slides were reviewed for histologic type according to the criteria mentioned by World Health Organization (WHO) [13] histological grade which evaluated using the Nottingham-combined histologic grade [Elston-Ellis modification of Scarff-Bloom-Richardson (SBR) grading system] [14]. Staging of tumors was carried out according to the American Joint Committee on Cancer (AJCC) TNM staging system of breast cancer, 7th edition [15].

Immunohistochemical methods.

All immunohistochemical analyses were performed on routinely processed, formalin-fixed, paraffin-embedded tissues. Antibodies used were: Primary monoclonal antibodies against CK5/6 (Dako, mouse monoclonal, clone D5/16B4, ready to use), EGFR (Cell Marque, Rabbit monoclonal, clone SP84, ready to use) and Claudin 4 (Thermo fisher, mouse monoclonal, clone 3E2C1, ready to use). Three unstained sections were prepared on positively charged slides for the assessment of CK5/6, EGFR, and Claudin 4.

Positive control slides were used within each batch of slides (surface squamous epithelium of skin piece for CK5/6 as well as EGFR, and section of ovarian carcinoma for Claudin 4), the thickness of paraffin sections were made 4 microns and mounted on positive charged slides, then immunostaining was done using BenchMark XT (Ventana) autostainer following protocols instruction.

Assessment of immunostaining:

CK5/6: Immunoreaction was positive when brown, homogenous cytoplasmic or membranous staining of tumor cells was seen. Semiquantitative method were used including, 0: For cases with no immunostaining, 1+: For positive immunostaining in less than 10%, 2+: For positive immunostaining in 10% to 50%, and 3+: For strongly positive immunostaining in >50%.

The scores 0 and 1 were considered as negative while scores 2 (Fig. 1A) and score 3 (Fig. 1B) were considered as positive [16].

EGFR: Clear staining of the tumor cell membranes was scored positive. Semiquantitative approach was used to generate a score for each tumor tissue sample: The percentage of positive tumor cells per slide (0%-100%) was multiplied by dominant intensity pattern of staining (1, negative or trace; 2, weak; 3, moderate; 4, intense). The overall score ranged from 0 to 400 and specimens were

considered having levels of EGFR expression: Negative or low (0-200) (considered negative), intermediate (201-300) (Fig. 2A) and high (301-400) (Fig. 2B) (intermediate and high considered positive) [17].

Claudin 4: Only membranous staining was classified as positive. Scoring was done according

to intensity of expression on a scale of 0-3 where 0 is negative, 1 is weakly positive, 2 is intermediate positive and 3 is strongly positive. High claudin-4 was classified as tumors with a staining intensity of ≥ 2 (Fig. 3A), and low claudin-4 was classified as tumors with a staining intensity of < 2 (Fig. 3B) [18].

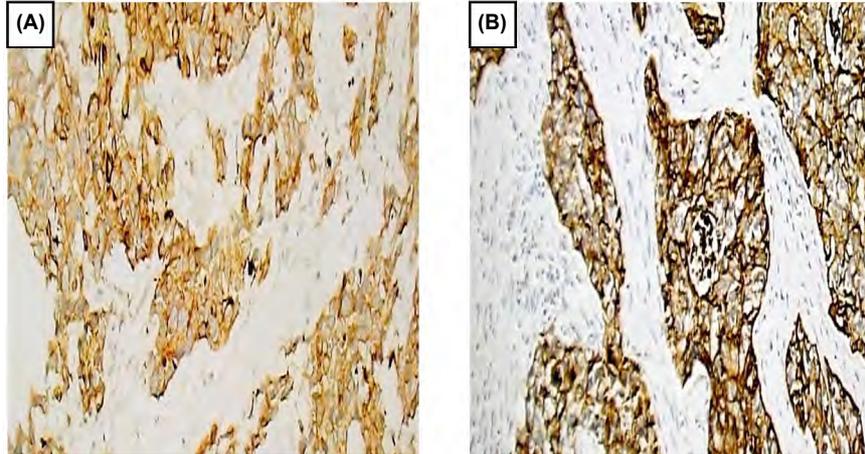


Fig. (1A): IHC to CK5/6, showing moderate cytoplasmic staining score 2 (X200). (B) IHC to CK5/6, showing strong cytoplasmic staining score 3, (X200).

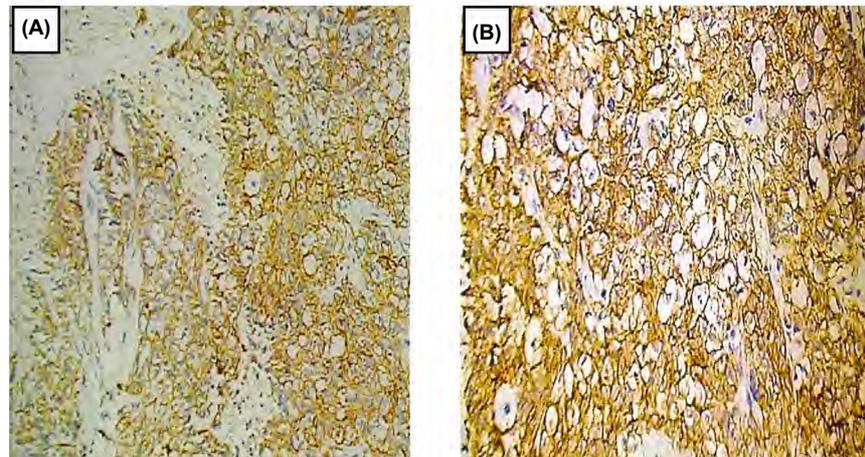


Fig. (2A): IHC to EGFR, showing intermediate membranous staining (x200). (B) IHC to EGFR, showing strong membranous staining (X200).

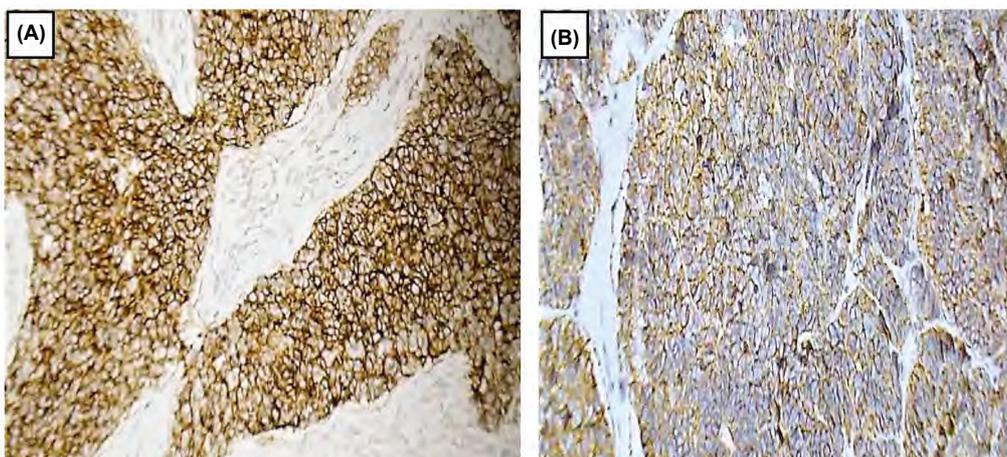


Fig. (3A): IHC to Claudin 4, showing membranous staining, score 3 [high claudin 4] (X200). (B) IHC to Claudin 4, showing no membranous staining, score 0 [low claudin 4] (X200).

Statistical methods:

Data was analyzed using IBM SPSS advanced statistics (Statistical Package for Social Sciences), version 22 (SPSS Inc., Chicago, IL). Numerical data was described as median and interquartile range or range or mean and standard deviation as appropriate, while qualitative data were described as number and percentage. Chi-square (Fisher's exact) test was used to examine the relation between qualitative variables as appropriate.

Survival analysis was done using Kaplan-Meier method. Comparison between two survival curves was done using log rank test. Multivariate analysis was done by Cox regression model to test for independent prognostic effect of statistically significant variables on univariate level with calculating hazard ratio and its 95% confidence interval. p -value < 0.05 was considered significant and all test was 2 tailed. Bonferonni corrections of p -value was done to avoid hyperinflation of type 1 error which arises from multiple testing.

A p -value less than or equal to 0.05 was considered statistically significant. All tests were two tailed.

Overall survival (OS) was calculated from date of diagnosis till date of death or last follows-up.

Disease free survival (PFS) was calculated from date of surgery till date of relapse, death or last follows-up.

Results

All 59 cases were included in the study were females between the age group 26 to 77 years with the mean age of 48.95 years, and median age was 50 years. All cases were IDC (NST). Most cases were postmenopausal, with negative family history. The majority of cases represented grade 2, T2, stage II, and with positive lymph nodes.

Results of immunohistochemical markers:

Most of cases were positive to CK5/6 (71.2%), with majority of cases represented Claudin 4 high (89.8%), while (45.8%) were positive to EGFR.

As regards relation with clinicopathologic parameters, there was a statistically significant relation obtained between CK 5/6 and size of the tumor as well as EGFR and stage of tumor. Relation of immunohistochemical markers and clinicopathologic parameters are listed in (Table 1).

Table (1): Relation of Immunohistochemical markers and clinicopathologic parameters.

Clinicopathological parameters	CK5/6		p -value	EGFR		p -value	Claudin 4		p -value
	Negative n (17)	Positive n (42)		Negative n (32)	Positive n (27)		Negative n (6)	Positive n (35)	
Age (years):									
<=50	11 (64.7%)	23 (54.8%)	0.484	21 (65.6%)	13 (48.1%)	0.176	4 (66.7%)	30 (56.6%)	1.000
>50	6 (35.3%)	19 (45.2%)		11 (34.4%)	14 (51.9%)		2 (33.3%)	23 (43.4%)	
Menopausal status:									
Pre	11 (64.7%)	17 (40.5%)	0.091	18 (56.2%)	10 (37.0%)	0.141	3 (50.0%)	25 (47.2%)	1.000
Post	6 (35.3%)	25 (59.5%)		14 (43.8%)	17 (63.0%)		3 (50.0%)	28 (52.8%)	
Family history:									
Positive	2 (11.8%)	5 (11.9%)	1.000	3 (9.4%)	4 (14.8%)	0.692	1 (16.7%)	6 (11.3%)	0.701
Negative	15 (88.2%)	37 (88.1%)		29 (90.6%)	23 (85.2%)		5 (83.3%)	47 (88.7%)	
Size:									
T1&T2	11 (64.7%)	38 (90.5%)	0.017	25 (78.1%)	24 (88.9%)	0.272	5 (83.3%)	44 (83.0%)	1.000
T3&T4	6 (35.3%)	4 (9.5%)		7 (21.9%)	3 (11.1%)		1 (16.7%)	9 (17.0%)	
Node status:									
Positive	11 (64.7%)	26 (61.9%)	0.84	23 (71.8%)	14 (51.8%)	0.784	5 (83.4%)	32 (60.4%)	0.396
Negative	6 (35.3%)	16 (38.1%)		9 (28.2%)	13 (48.2%)		1 (16.6%)	21 (39.6%)	
Grade:									
1&2	10 (58.8%)	27 (64.3%)	0.694	19 (59.4%)	18 (66.7%)	0.564	4 (66.7%)	33 (62.3%)	1.000
Grade 3	7 (41.2%)	9 (35.7%)		13 (40.6%)	9 (33.3%)		2 (33.3%)	20 (37.7%)	
Stage:									
1&2	9 (52.9%)	29 (69.0%)	0.242	17 (53.1%)	21 (77.8%)	0.049	4 (66.7%)	34 (64.2%)	1.000
3&4	8 (47.1%)	13 (31.0%)		15 (46.9%)	6 (22.2%)		2 (33.3%)	19 (35.8%)	
Metastasis:									
Positive	3 (17.6%)	6 (14.3%)	0.745	6 (18.8%)	3 (11.1%)	0.488	1 (16.7%)	8 (15.1%)	0.919
Negative	14 (82.4%)	36 (85.7%)		26 (81.2%)	24 (88.9%)		5 (83.3%)	45 (84.9%)	
Recurrence:									
Positive	0 (0.0%)	5 (11.9%)	0.308	3 (9.4%)	2 (7.4%)	*	1 (16.7%)	4 (7.5%)	*
Negative	17 (100%)	37 (88.1%)		29 (90.6%)	25 (92.6%)		5 (83.3%)	4 (92.5%)	
Outcome:									
Good	13 (76.5%)	30 (71.4%)	0.693	24 (75.0%)	19 (70.4%)	0.690	3 (50.0%)	40 (75.5%)	0.330
Bad	4 (23.5%)	12 (28.6%)		8 (25.0%)	8 (29.6%)		3 (50.0%)	13 (24.5%)	

* p -value cannot be stated because of small number within strata.

The relation of positivity of immunohistochemical markers with each other revealed a tendency for cases positive for CK 5/6 to be positive also for EGFR, yet this relation did not reach the point of statistical significance.

According to survival analysis, there is a significant relationship found between OS with grade, stage, occurrence of metastasis and node positivity (Table 2) as well as between grade and DFS (Table 3).

Table (2): Overall survival and its relation to clinicopathologic parameters and Immunohistochemical markers.

	No.	No. of events	Cumulative survival at 12 months (%)	Cumulative survival at 36 months (%)	Cumulative survival at 60 months (%)	<i>p</i> -value
<i>Whole group:</i>	59	11	96.6	84.7	79.7	–
<i>Age:</i>						
< 50	34	4	97.1	93.9	84.9	0.089
>50	25	7	92.0	72.0	72.0	
<i>Menopausal status:</i>						
Pre	28	3	100.0	96.4	85.9	0.115
post	31	8	93.5	74.2	74.2	
<i>Family history:</i>						
Positive	7	2	85.7	71.4	71.4	0.473
Negative	52	9	96.2	86.5	80.9	
<i>Grade:</i>						
Grade 1 &2	37	10	94.6	78.2	68.8	0.026
Grade 3	22	1	100.0	95.5	95.5	
<i>Tumor size:</i>						
T1 &T2	49	7	95.9	87.7	84.8	0.070
T3 &T4	10	4	90.0	70.0	58.3	
<i>Stage:</i>						
Stge 1&2	38	9	100.0	94.7	91.2	0.003
Stge 3&4	21	8	90.5	66.7	60.0	
<i>LN status:</i>						
Positive	37	10	94.6	78.2	69.6	0.031
Negative	22	1	100.0	95.5	95.5	
<i>Metastasis:</i>						
Positive	9	6	100.0	55.6	NR	<0.001
Negative	50	5	96.0	89.9	89.9	
<i>CK5/6:</i>						
Positive	42	8	97.6	83.3	79.4	0.905
Negative	17	3	94.1	88.2	80.2	
<i>EGFR:</i>						
Positive	27	4	96.3	85.2	85.2	0.603
Negative	32	7	96.9	84.3	76.2	
<i>Claudin 4:</i>						
High	53	9	96.2	84.8	81.6	0.408
Low	6	2	100.0	83.3	66.7	

NR: Not reached.

Table (3): Disease free survival and its relation to clinicopathologic parameters and Immunohistochemical markers.

	No.	No. of events	Cumulative survival at 12 months (%)	Cumulative survival at 36 months (%)	Cumulative survival at 60 months (%)	Median survival estimate	p-value
<i>Whole group:</i>	59	20	100	92.5	67.9	67.900	–
<i>Age (years):</i>							
≤50	34	10	100	93.8	82.0	67.900	0.922
>50	25	10	100	95.7	53.8	76.900	
<i>Menopausal status:</i>							
Pre	28	9	100	96.2	75.4	66.900	0.764
post	31	11	100	89.3	61.4	76.900	
<i>Family history:</i>							
Positive	7	4	100	100	75.5	64.900	0.757
Negative	52	16	100	91.3	67.0	72.900	
<i>Grade:</i>							
Grade1&2	37	17	100	88.4	60.5	66.900	0.035
Grade 3	22	3	100	100	85.7	77.900	
<i>Tumor size:</i>							
T1&T2	49	15	100	90.9	67.2	66.900	0.173
T3&T4	10	5	100	100	71.4	75.900	
<i>Stage:</i>							
Stge 1&2	38	11	97.4	94.6	69.3	66.900	0.852
Stge 3&4	21	9	100	89.5	66.1	75.900	
<i>LN status:</i>							
Positive	37	17	100	91.1	65.6	66.900	0.276
Negative	22	3	100	95.5	27.7	*	
<i>CK5/6:</i>							
Positive	42	14	100	92.0	71.9	72.900	0.795
Negative	17	6	100	93.8	57.3	66.900	
<i>EGFR:</i>							
Positive	27	10	100	98.5	60	64.900	0.261
Negative	32	10	100	89.5	76.9	75.900	
<i>Claudin 4:</i>							
High	53	17	100	93.7	68.6	64.900	0.200
Low	6	3	100	83.3	62.5	72.900	

Discussion

Many studies revealed that triple negative breast cancer is a heterogeneous group of breast cancer [19]. Basal markers such as CK5/6 and EGFR as well as Claudin 4 are considered important in subclassification of the triple negative group and can also affect treatment options [20,21].

In our study most of cases positive to CK5/6 were < or equal 50 years. Cases expressed CK5/6 were characterized by T2 size (71.4%), showing statistically significant relationship between expression of CK5/6 and tumor size (p -value 0.017). Most of the positive cases are of grade II (64.3%), followed by grade III (35.7%) and none is of grade I. The greatest percentage of node positive status is among positive group (61.9%). This aggressive course was reflected on the stage of the CK 5/6 positive cases with stage 2 represented (61.9%) followed by stage 3 (28.5%), and the only case of stage 4, belonged to the CK 5/6 positive group,

with all the cases who underwent recurrence and 6/9 cases who underwent metastasis are CK5/6 positive. Similar findings were reported by Preethi and colleagues, with range of age at presentation among CK 5/6 positive patients was 41-58 years, commonest size was T2, commonest grade is grade 2 (57.2%) followed by grade 3 (42.8%), and 50% showed node metastasis [16]. Nearly similar results were also reported in other studies [22].

As regards EGFR (45.8%) represented positive cases. The relation of EGFR with clinicopathologic parameters revealed that stage II was the commonest stage, with significant relationship with stage and expression of EGFR (p -value 0.049). Most of cases positive to EGFR were more than 50 years, of grade II tumors (66.7%) of T2 (77.8%), and with node positivity (51.8%). Only three cases underwent metastasis, and two underwent recurrence, with no significant relationships detected with any of these parameters.

Discrepant results are retrieved from various studies as regards prognostic impact of EGFR on TNBC. Some studies agreed with ours as Sobande and colleagues who reported no significant relationship between expression of EGFR with grade and node positivity denoting that EGFR changes did not appear to be associated with markers of aggressive behavior in TNBC [23]. Others reported bad prognosis of EGFR positive cases [22].

To reach a definite conclusion about prognostic impact of the expression of EGFR a lot of studies with large sample size are intensively needed, to justify the routine implication of this marker for stratification of patients with TNBC and to identify candidates who could benefit from its targeted therapy.

Minority of cases represented Claudin low group which were only six cases (10.2%), which is similar to many studies denoted that Claudin low group revealed minor cases among TNBC [24,25].

Claudin high type was reported in 89.8% of our cases. Most cases were of age ≤ 50 . Cases of T2, T3, and all cases of T4, most high grades (2&3), high stages, and node positivity were all Claudin high, however, none of these parameters reached statistically significant relation. Eight cases underwent metastasis from total nine in this study and four cases underwent recurrence from total five are of Claudin high group. Abd-Elazeem and Abd-Elazeem who studied also Egyptian patients reported similar figures as most of cases of Claudin high were less than 50 years, showed tumor size T2 and T3, high grade, high stage, and node positivity [26]. Also similar results were reported by Ko and colleagues [27]. These results point to that high expression of Claudin 4 may be associated with poor prognosis and would provide a strong prognostic power to differentiate the patients with worse outcome among triple negative breast cancer patients. Kolokytha and colleagues reported that increased immunohistochemical expression of Claudin-4 was found to be related to favorable prognostic parameters among triple negative but not luminal type breast cancer [10]. This highlights that expression of the same protein could exert different prognostic impact among different molecular subtypes of breast cancer. Also ethnic factors may play role in different impact.

Although Claudins act to increase cell to cell adhesions and thus expected for tumors over expressing them to behave in a less aggressive man-

ner, our results are actually the opposite. An assumption that among triple negative molecular subtype, different signaling pathways could interfere with post-translational modification of the expressed Claudin 4, could explain this controversy [10] More trails and studies concerning Claudin 4 and its relation to different molecular subtypes are needed to clarify the mechanism of action of this protein and the credibility of the administration of targeted therapy with the use of anticlaudin antibodies.

A significant relationship was found between the disease free and overall survival with grade of the tumor, which revealed the effectiveness of the chemotherapy treatment on the higher grade tumor with improvement of the survival, with similarity to many studies [28].

Finally, we concluded that among Egyptian patients, triple negative breast cancer did not comprise one disease group. It could be better stratified according to the expression of different immunohistochemical markers which could help in decision making as regards enrollment in clinical trials and application of new therapeutic agents targeting the different expressed proteins by the tumor. Also, the different results we obtained as regard prognostic impact of EGFR and Claudin 4 from other literature is an interesting and encouraging point to further study these markers on Egyptian population and clarify if there is statistically significant difference which could be attributed to ethnic factors, also concluded that the well established traditional factors which are the clinicopathologic parameters still play the most crucial role in prognosis, other than the markers which need more studies on large scale of cases.

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دراسة مجموعة من الصبغات المناعية على المرضى المصريين بحالات سرطان الثدي الثلاثى السلبية لمحاولة تقسيمها

سرطان الثدي الثلاثى السلبية من مجموعات سرطان الثدي التى تخضع لعدة دراسات لمحاولة تقسيمها إلى مجموعات صغيرة لإيجاد وسائل علاج مناسبة لكل مجموعة.

الهدف من الدراسة: دراسة وأجراء مجموعة من الصبغات المناعية على حالات سرطان الثدي الثلاثى السلبية لمحاولة تقسيمها إلى عدة مجموعات أخرى.

طرق البحث: أجرى البحث على ٥٩ حالة من حالات سرطان الثدي الثلاثى السلبية، تم تشخيصهم فى قسم الباثولوجى فى معهد الأورام جامعة القاهرة، استخدام مجموعة من الصبغات المناعية على هذه الحالات وهى سيتو كيراتين ٦/٥، أيه جى أف آر وكولدين ٤ وكان مترسب زمن متابعة الحالات هو أربعون شهر ونصف.

نتائج البحث: كانت الحالات الإيجابية للسيتو كيراتين ٦/٥، تمثل ٧١.٢٪ مع وجود علاقة إحصائية قوية بين الإيجابية للسيتو كيراتين ٦/٥ وحجم الورم، والحالات الإيجابية للأيه جى أف آر تمثل ٤٥.٨٪ مع وجود علاقة إحصائية قوية بينه وبين درجة الورم، ٨٩.٨٪ يمثلون مجموعة الأكثر إيجابية للكولدين ٤، معظمهم ذو درجة ورم عالية، مرحلة ورم عالية وانتشار أكثر للغدد الليمفاوية، لكن لم نحصل على دلالة إحصائية قوية مع أى من العوامل الباثولوجية والكلينيكية. ووجود علاقة إحصائية قوية بين درجة الورم والعيش لفترة أطول دون الأرتجاع وأيضاً علاقة قوية بين درجة الورم، مرحلة الورم والانتشار إلى الغدد والانتشار إلى أماكن أخرى مع العيش لفترة أطول.

الاستنتاج: مجموعة سرطان الثدي الثلاثى السلبية مجموعة مختلطة تحتاج إلى مجموعة من الصبغات المناعية مثل سيتو كيراتين ٦/٥، أيه جى أف آر وكولدين ٤ لتقسيمها إلى مجموعات صغيرة لإيجاد العلاج الأمثل والأنسب لكل مجموعة.