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RESEARCH ARTICLE

High expression levels of both E-Cadherin and Ki-67 in triple negative breast cancer tissues corerlate with positive nodal metastasis

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ABSTRACT

Background: Breast cancer is one of the most diverse and well-known diseases. It has various molecular subtypes, clinical behaviors, therapeutic responses, and patient outcomes. The absence of estrogen receptor (ER) and progesterone receptor (PR) expression and the lack of excessive human epidermal growth factor receptor 2(HER2) expression are the two characteristics that are often used to identify triple-negative breast cancer (ER-/PR-/HER2-). E-Cadherin is a cell adhesion molecule that inhibits metastasis, invasion, and cell growth. A non-histone nuclear protein called Ki-67 is connected to tissue and cellular proliferation. **Aim: This study was designed to** evaluate the pattern of expression of E-cadherin and Ki-67 in the studied triple-negative breast cancer cases and to find their correlation with various clinicopathological parameters. **Patients and Methods:** This study was carried out on 60 cases of triple-negative breast cancer. Immunohistochemical staining using E-cadherin and Ki-67 antibodies was done for all cases to evaluate their pattern of expression. **Results:** There was a significant statistical negative relation between E-cadherin expression in TNBC and pleomorphic lobular subtype and axillary lymph node metastasis. Also, there was a significant statistical relation between high Ki-67 index (P value < 0.05) and high-grade histological types of TNBC cases, age groups and cases from lower inner quadrant. **Conclusion:** Decreased E-cadherin expression is related to positive nodal metastasis. High tumor grade was substantially correlated with high levels of Ki-67 expression.

Keywords: Cell adhesion molecule, E-Cadherin; Ki-67; Non-histone nuclear protein, Triple Negative Breast Cancer

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INTRODUCTION

Breast cancer is one of the most diverse and well-known diseases. It has various molecular subtypes, clinical behaviors, therapeutic responses, and patient outcomes (Ricciardi et al., 2015). To well manage this disease, it is very important to identify these subtypes as it is a challenge to accurately predict the prognosis and decide the best treatment regimen to use for each individual case (Ameh-Mensah et al., 2021).

The absence of estrogen receptor (ER) and progesterone receptor (PR) expression and the lack of excessive human epidermal growth factor receptor 2 expression and/or amplification by fluorescence in situ hybridization (FISH) or immunohistochemistry (IHC) are the two characteristics that are often used to identify triple-negative breast cancer (HER2). TNBC is a clinical phenotype that is aggressive and makes up 10% to 20% of all breast cancer. Endocrine treatment and trastuzumab-based HER2-targeted therapy are ineffective for TNBC patients (Jing et al., 2020).

A transmembrane glycoprotein called E-cadherin facilitates calcium-dependent cell-to-cell attachment. It plays a crucial role in the development of a polarized epithelial phenotype. It features a cytoplasmic domain that connects to regulatory proteins such as catenin. A crucial function of the E-cadherin/b-catenin complex is the preservation of epithelial integrity.

Moreover, this complex's disruption is related to a wide variety of malignant tumors because of the process of epithelial-mesenchymal transition (EMT) (Shen et al., 2016).

Inactivating mutations of β-catenin and E-cadherin encoding genes was discovered in most epithelial tumors which led to E-cadherin function loss. As a result, the epithelial tumors acquire the ability to invade and spread. (Ali et al., 2020) The downregulation of E-cadherin expression is a defining characteristic of the epithelial-to-mesenchymal transition. (Ali et al., 2020)

Since Ki-67's expression changes throughout the cell cycle with a peak during mitosis, it is often utilized as a proliferative marker. Although Ki-67's significance as a prognostic and predictive marker has been extensively explored in BC, there is currently no agreed-upon cut-off criteria, and its precise role is still unknown (Zhu et al., 2020). However, there is a tight association between Ki-67 and cell-cycle phase regulation. Furthermore, because of its short half-life, it is a reliable marker for determining the proportion of neoplastic cell populations that are growing. (Ricciardi et al., 2015) Currently, it is recommended in common practice to quantify the amount of Ki-67 expression to gauge the rate of cell proliferation in breast cancer. In ER+/Her2- BC, it is primarily utilized to assess prognosis, direct adjuvant therapy, and forecast the response to neo-adjuvant therapy; however, its use in TNBC is still unclear (Focke et al., 2017).

The aim of this study was to evaluate the significance of E-cadherin and Ki-67 expression in TNBC and their correlation with clinic pathological parameters.

PATIENTS AND METHODS

This retrospective study was carried out on 60 cases of TNBC. The cases were collected from archive of Pathology department, Faculty of Medicine, Tanta University, Egypt and some private laboratories during the period from August 2019 to May 2021. The cases' parents or guardians (are the cases children?) were given written, fully informed permission.' The study was approved by Research Ethics Committee (REC), Faculty of Medicine, Tanta University, Egypt with permission number 33903/6/20. Specimens were received as paraffin blocks. Sections were prepared for routine hematoxylin and eosin (H&E) staining (for re-evaluation) and immunohistochemical staining by E-cadherin and Ki-67 antibodies.

Clinical Findings: Clinic-pathological data was collected from patients' surgical and radiological reports. Age of the patient at diagnosis was classified into less than or equal to 50 years and more than 50 years. All cases were females. Tumor size was classified into two groups according to AJCC TNM staging system: A. tumors with size < 5 cm including T1 (1mm-20 mm) and T2 (20-50 mm) and B. Tumors with size more than 5cm including T3 (>5cm) and T4 (any size with direct extension to chest wall and /or to the skin) (Giuliano et al., 2018). The side and site of the tumor was obtained from pathology request of operative sheet and the available gross specimens. The samples were obtained from modified radical mastectomy (MRM), CBS and quadrantectomy specimens. The gross picture of each case is obtained from pathology reports or available specimens. All collected cases were greyish-white and firm with speculated margins, some showed necrosis, hemorrhage, and cystic degeneration.

Histopathological Evaluation
Sections from the studied cases were subjected to hematoxylin and eosin staining

For histopathological examination, all cases were examined using a light microscope Leica. The histopathological type was determined according to WHO classification (5th edition) of Tumors of the Breast 2019 (Tan et al., 2020)**.**

The histological grade was determined according to Nottingham modification of the Bloom–Richardson system (Pradhan et al., 2017). Carcinoma associated with in situ component, lympho-vascular invasion, peri-neural infiltration, necrosis, and lymphocytic background were evaluated for each case. Lymph node status was classified into: A. cases without L. N metastasis (N0) and B. cases with L.N metastasis (N1, N2, N3).

Procedure of immunohistochemical staining: On positively charged slides, 5 m thick tumor

slices were dried for 30 minutes at 37 C. Deparaffinization of the sections was followed by antigen retrieval with high and low PH EnVision FLEX antigen retrieval solutions in a Dako PT link unit (97°C for 20 min). Dako Autostainer Link 48 was used to accomplish immunostaining. Shortly after applying the peroxidase blocking reagent, the primary antibodies were incubated for 30 minutes. Following that, diaminobenzidine (DAB) was used as the chromogen and horseradish peroxidase polymer was applied for 20 minutes. Hematoxylin was used as a counterstain on the slides (Mohamed and Abo Safia, 2020).

The primary antibodies used in this study:

1. E-cadherin mouse monoclonal antibody IgG1/Kappa, ready for usage, offered in liquid state in a buffer with stabilizing protein and 0,015 mol/L NaN3.
2. Ki-67 mouse monoclonal antibody IgG1/Kappa, ready for usage, offered in liquid state in a buffer with stabilizing protein and 0,015 mol/L NaN3 sodium aside. Control slides: Positive control; for E-cadherin was a section in DCIS of the breast (Figure 1a). For Ki-67: was a section in small cell carcinoma of the lung (Figure 1b).

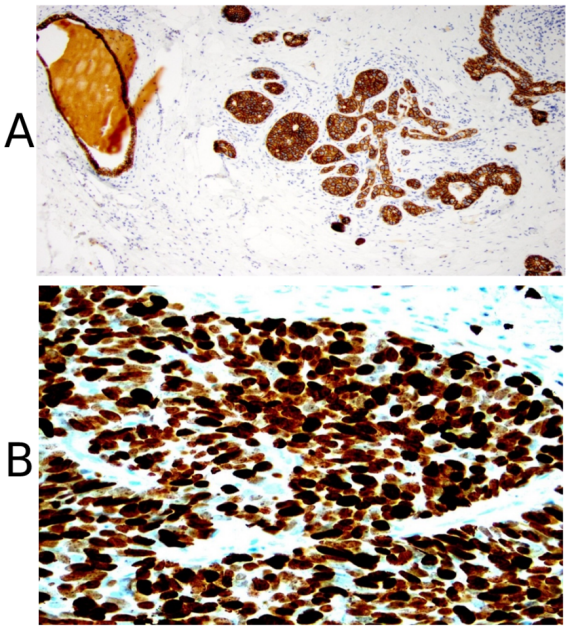

Figure 1: A) A case of ductal carcinoma in situ of the breast showing strong membranous expression of E-cadherin (streptavidin biotin, x 200). B) A case of small cell carcinoma of the lung. Immunohistochemical stain for Ki67 shows > 80% positive staining in the tumor cell nuclei (streptavidin biotin, x 200).

Immunohistochemical examination: Hormone receptor status: obtained ER, PR, and HER2 immunostained slides were re-examined and re-evaluated. Evaluation of PR and ER: They were identified as nuclear staining-positive tumor cells. The final score ranges from 0 (no tumor staining) to 100 (complete staining) (diffuse strong staining). If a tumor's ER and PR scores were more than 10%, it was deemed hormone receptor positive. (Iqbal and Buch, 2016). Scoring of HER2 IHC assay: A semi-quantitative approach with a score range of (0-3) was adopted, depending on the degree of membrane staining and the proportion of membrane-positive cells. Scores 0 (negative) indicate no staining or membranous staining in excess of 10%, 1 (negative) membranous staining that is faint or incomplete in excess of 10%, 2 (positive/borderline) weak to moderate complete staining in excess of 10%, and 3 (positive) strong complete membranous staining in excess of 10%.

Interpretation and scoring of E-cadherin: (Alaa Edin et al., 2021) The proportion of stained cells was determined using a semi-quantitative approach using the following scoring criteria:

- <5%, 0 points, (0);
- 5-25%, 1 point, (1);
- 26-50%, 2 points, (2);
- 51-75%, 3 points, (3);
- >75%, 4 points, (4).

In accordance with manufacturer's guidelines, membranous or membranous and cytoplasmic staining was regarded as positive. Staining intensity (SI) was also considered in evaluating the IHC expression of E-cadherin. It receives a score of 1, 2, or 3, depending on how strongly stained it is. Calculating immunohistochemical scores (IHCS) included multiplying the staining intensity (SI) by the proportion of positive cells (PP). IHCS= PP X SI was therefore employed as the formula. So, final E- cadherin scoring was:

- Negative 0
- Mild 1-2
- Moderate 3-4-6
- Marked 8-9-12

Interpretation of Ki-67: (Nielsen et al., 2021) According to Ki67-QC international working group: procedure for whole-section scoring

(global method), Ki67 index was interpreted through multi-step system as follow:

Step 1: specifying the percentages: (By low-power) After excluding carcinoma in situ and non-tumor tissue like necrosis and fibrosis, the proportions of the invasive tumor on the glass slide were estimated and graded as follows: Negative, that is, invasive cells are present but the fraction of positive invasive cells is very low (or even nonexistent), low, medium, or high, as identified previously.

Step 2: choosing the appropriate fields: We may calculate the number and staining classification (high/medium/low/negative) of needed fields (up to 4) to score based on the percentage of Ki67 representation stated in the preceding step, then record the location of the chosen fields.

Step 3: grading the representative fields: For every one of the necessary fields chosen in step 2, place the high-powered (40x objective) microscope field in the vicinity of the chosen scoring field. Next, count the number of invasive tumour nuclei in a "typewriter" pattern starting from the top of the chosen scoring field, stopping when either 100 invasive tumor nuclei have been counted overall or all invasive tumor nuclei in the chosen scoring field have been counted, whatever comes first.

Step 4: determining final Ki-67 score: Following is how the final Ki67 score was determined:

$Ki67\ score$ = ($total$ number of + $ve\ tumor\ nuclei\ counted\ in\ all\ fields$/ $total$ number $of\ tumor\ nuclei\ counted\ in\ all\ fields$) × 100

On the basis of percentage of staining, ki67 index was further categorized into four groups, very low < 14%, low 14–24%, intermediate 25–45%, high >45%.

Statistical analysis

SPSS software was used to arrange, tabulate, and statistically analyze the obtained data (Statistical Package for the Social Sciences, version 21, SPSS Inc. Chicago, IL, USA).

Categorical data were evaluated by chi-square test and when it was inappropriate it was replaced by Fischer Exact or Monte Carlo Exact test. The Pearson correlation coefficient (r) or Spearman correlation was used to assess the correlation between the variables (rs). P value was deemed significant when it was below 0.05. For the sake of interpreting the findings of significance tests, significance was set at $p < 0.05$.

RESULTS

Clinicopathologic characteristics of triple negative breast carcinoma cases

The current retrospective study included 60 cases of TNBC. The mean age of the studied cases was 58.8 years (range, 39–77 years). Tumors from the left breast represented 55% of cases. Also, 45% of cases were from the upper outer quadrant while 5% of cases were from the lower inner quadrant. Most of cases 60% were T2 as illustrated in Table 1.

Histopathological results

Most of the studied TNBC cases 20% were metaplastic carcinoma, while invasive ductal carcinoma with medullary features cases represented 15%.

Pleomorphic lobular carcinoma constituted 5% of cases. Lympho-vascular invasion was detected in 36% of cases while perineural infiltration was present in 26.7% of cases. Also, intraductal component was found in 20% of cases. Forty percent of cases showed tumor necrosis and lymphocytic background were also detected in 40% of cases. Most of cases 65% were grade III, while 5% of cases where grade I. Nodal metastasis was present in 70% of cases as follow 26.7%, 30%, 13.3% for N1, N2 and N3 respectively as illustrated in Table 1 and Figure 2.

Immunohistochemical results

E-cadherin immunohistochemical results

Table 2 and Figure 3 show the relation between E-Cadherin expression and histological types. There was a significant negative statistical relation between expression of E-cadherin in TNBC and pleomorphic lobular subtype. Also, there was a strong significant negative statistical relation between expression of E-cadherin in TNBC and axillary lymph node metastasis. But there was no significant statistical relation between expression of E-cadherin in TNBC and age groups in the study group (with P value =0.365).

Table 1. Age distribution, side, size, and site of tumor, histological types, vascular invasion, perineural infiltration, intra ductal component, tumor necrosis, lymphocytic background, grades, nodal status, and T staging of the studied cases.

		Number of cases	Percent
Age groups	A ≤ 50	25	41.7%
	B > 50	35	58.3%
Side	Right breast	27	45.0%
	Left breast	33	55.0%
Site	Upper outer quadrant	27	45.0%
	Lower outer quadrant	15	25.0%
	Central	9	15.0%
	Upper inner quadrant	6	10.0%
	Lower inner quadrant	3	5.0%
Size	T1 (<2cm)	6	10.0%
	T2 (2-5 cm)	36	60.0%
	T3 (>5 cm)	18	30.0%
Histological types	Invasive Ductal Carcinoma –NST	24	40.0%
	Invasive Ductal Carcinoma with medullary features	9	15.0%
	Pleomorphic lobular carcinoma	3	5.0%
	Mixed IDC & ILC	6	10.0%
	Metaplastic carcinoma	12	20.0%
	Adenoid cystic carcinoma	6	10.0%
Vascular invasion	Present	22	36.7%
	Absent	38	63.3%
Perineural infiltration	Present	16	26.7%
	Absent	44	73.3%
Intra ductal in situ component	Present	12	20.0%
	Absent	48	80.0%
Tumor necrosis	Present	24	40.0%
	Absent	36	60.0%
Lymphocytic background	Present	24	40.0%
	Absent	36	60.0%
Grade	G I	3	5.0%
	G II	18	30.0%
	G III	39	65.0%
Nodal status	N0	18	30.0%
	N1	16	26.7%
	N2	18	30.0%
	N3	8	13.3%
T staging	T1	6	10.0%
	T2	36	60.0%
	T3	16	26.7%
	T4	2	3.3%
Total		60	100.0%

Also**,** there was no significant statistical relation between expression of E-cadherin in TNBC and tumor site, tumor grade in the study group (with P value =0.112 and 0.357) respectively.

Ki-67 immunohistochemical results

Table 3 and Figure 4 show the relationship between Ki-67 and histological types. There was a strong significant statistical relation between high grade histological types of TNBC cases and high KI-67 index. Also, there was a strong statistically significant relation between high index of Ki-67 in TNBC cases and ages groups. Moreover, there was a significant statistical relation between TNBC cases from lower inner quadrant and high Ki-67 index with P-value = 0.026*. But there was no significant statistical relation between nodal status in TNBC cases and KI-67 proliferative index with P-value (0.106). However, a strong significant statistical proportional relation was found between high grade TNBC cases and high Ki-67 proliferative index (P =0.002) as shown in Table 3.

Correlation between E-Cadherin, Ki-67 and different variables

Table 4 shows the correlation between E-cadherin & different variables. There were statistically significant weak negative correlations between E-cadherin

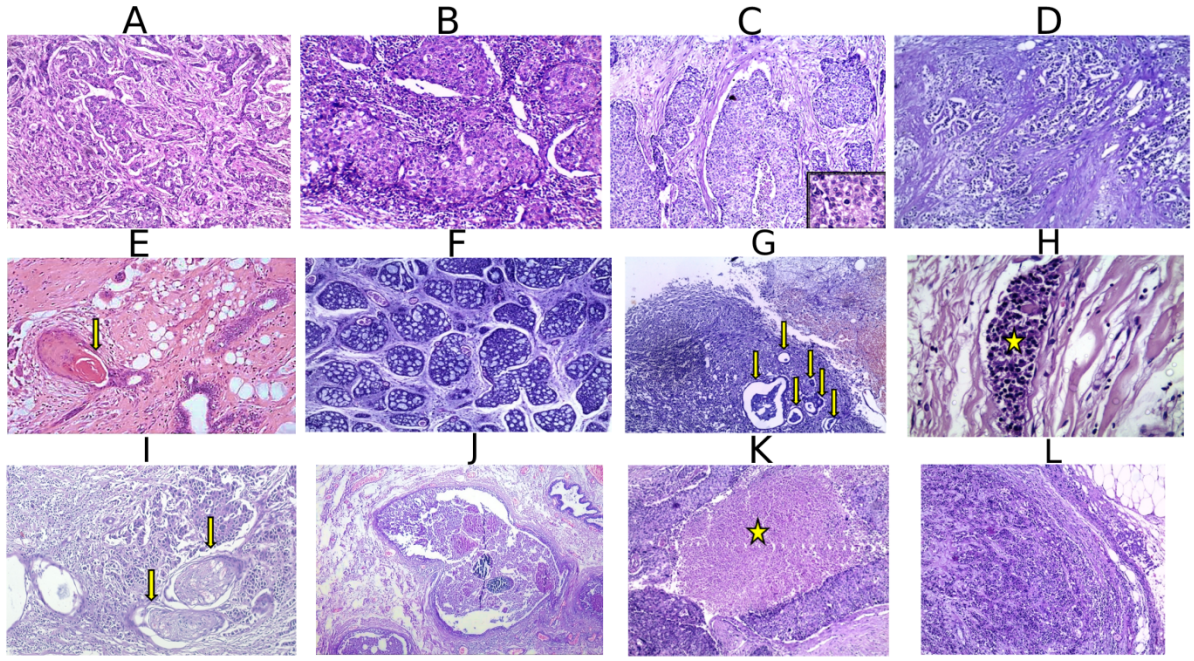

Figure 2. Histopathological findings (A) A case of IDC-NST showing tubule formation admixed with nests, cords & individual malignant ductal cells in desmoplastic back ground [H&E, x 200], (B) IDC with medullary features showing sheets of high grade ductal cells with pushing borders & intense peri tumoral & intertumoral lymphocytes [H&E, x200], (C) A case of IDC with medullary features showing sheets of high grade cells with prominent nucleoli and associated mitotic figures (star mitosis, inbox) [H&E, x200], (D) A case of pleomorphic lobular carcinoma showing tumor cells arranged in single file pattern and single cells with high degree of anaplasia in marked desmoplastic background [H&E, x100], (E) A case of metaplastic carcinoma malignant cells with squamous differentiation and keratin pearl formation (arrow) [H&E, x100], (F) A case of adenoid cystic carcinoma showing extensive cribriform architecture and infiltrative growth pattern [H&E,x100], (G) A case of metaplastic carcinoma showing vascular emboli; groups of malignant cells inside vascular lumens (arrows) [H&E, x100], (H) A case of mixed IDC & ILC showing vascular embolus; groups of malignant cells inside vascular lumen (star) [H&E, x400], (I) A case of IDC-NST showing neural invasion, groups of malignant cells surrounding & infiltrating sheath of the nerve bundle (arrows) [H&E, x200], (J) A case of IDC-NST showing intra ductal comedo type with calcification [H&E, x100], (K) A case of IDC with medullary features showing associated tumor necrosis (star) [H&E, x100], (L) Metastatic IDC in axillary lymph node [H&E, x100].

Table 2. Relation between E-Cadherin, and clinicopathologic parameters.

E-Cad group		Negative		Mild 1-2		Moderate 3-4-6		Marked 8-9-12		MC	P value
Histological types	IDC-NST	0	0 %	0	0 %	3	12.5 %	21	87.5 %	99.841	<0.001*
	IDC with medullary features	0	0 %	3	33.3%	3	33.3 %	3	33.3 %		
	Pleomorphic lobular carcinoma	3	100 %	0	0 %	0	0 %	0	0 %		
	Mixed IDC & ILC	0	0 %	3	50 %	3	50 %	0	0 %		
	Metaplastic carcinoma	0	0 %	3	25 %	9	75 %	0	0 %		
	Adenoid cystic carcinoma	0	0 %	0	0 %	3	50 %	3	50 %		
	Total	3	5 %	9	15 %	21	35 %	27	45 %		
Nodal status	N0	0	0 %	3	16.7%	5	27.8 %	10	55.6 %	22.641	0.006*
	N1	0	0 %	3	18.8%	3	18.8 %	10	62.5 %		
	N2	1	5.6 %	0	0 %	10	55.6 %	7	38.9 %		
	N3	2	25 %	3	37.5%	3	37.5 %	0	0 %		
	Total	3	5 %	9	15 %	21	35 %	27	45 %		
Tumor site	U.O.Q	3	11.1%	5	18.5%	10	37 %	9	33.3 %	17.898	0.112
	L.O.Q	0	0 %	4	26.7%	2	13.3 %	9	60 %		
	Central	0	0 %	0	0 %	6	66.7 %	3	33.3 %		
	U.I.Q	0	0 %	0	0 %	3	50 %	3	50 %		
	L.I.Q	0	0 %	0	0 %	0	0 %	3	100 %		
	Total	3	5 %	9	15 %	21	35 %	27	45 %		
Tumor grade	G Ⅰ	0	0 %	0	0 %	0	0 %	3	100 %	6.851	0.357
	G Ⅱ	0	0 %	2	11.1%	6	33.3 %	10	55.6 %		
	G Ⅲ	3	7.7 %	7	17.9%	15	38.5 %	14	35.9 %		
	Total	3	5 %	9	15 %	21	35 %	27	45 %		

MC: Monte Carlo Exact test *p ≤ 0.05 statistically significant

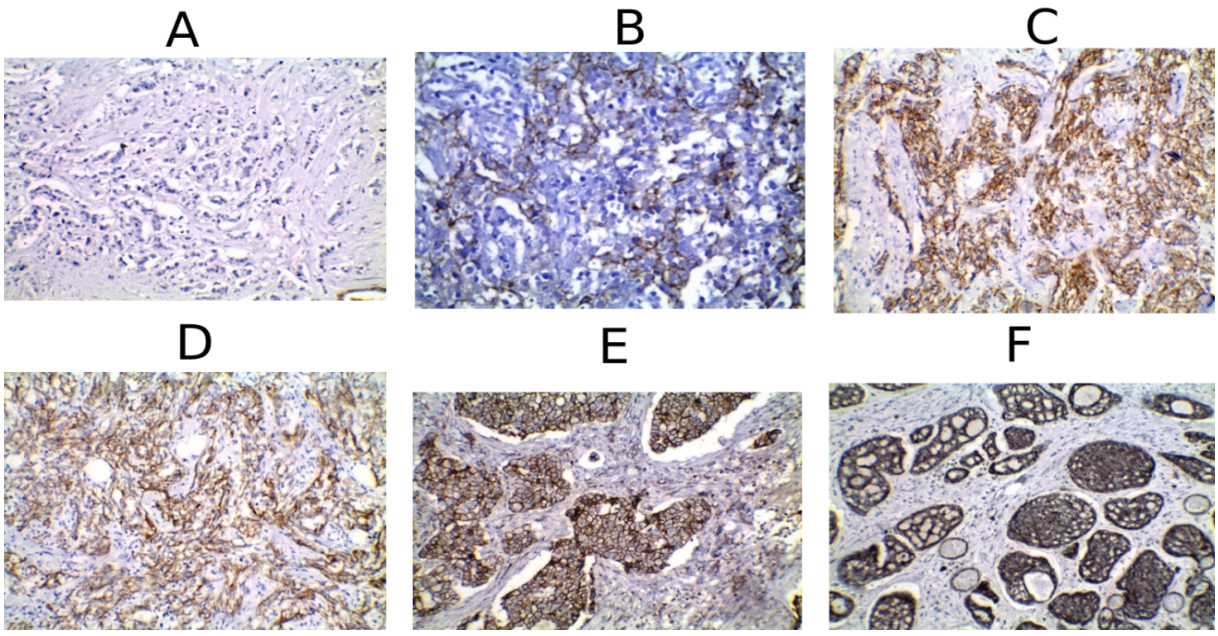

Figure 3. Immunohistochemical expression of E- cadherin. A) A case of pleomorphic ILC showing negative E-cadherin expression, score 0, (B) A case of metaplastic carcinoma showing moderate E-cadherin expression, score 3x1, (C) A case of high-grade IDC_NST showing high expression of E-cadherin, score 4x2, (D) A case of IDC with spindle cell component showing strong expression of E-cadherin, score 4x3, (E) A case of IDC with medullary features showing strong expression of E-cadherin, score 4x3, (F) A case of Adenoid cystic carcinoma showing strong expression of E-cadherin, score 4x3 [streptavidin biotin, x200]

Table 3. Relation between Ki-67, and clinicopathologic parameters

	Ki-67	Low 14 - 24		Intermediate 25 - 45		High > 45		MC	P value
Histological types	IDC-NST	1	4.2 %	2	8.3 %	21	87.5 %	24.252	0.009*
	IDC with medullary features	1	11.1 %	3	33.3 %	5	55.6 %		
	Pleomorphic lobular carcinoma	0	0 %	0	0 %	3	100 %		
	Mixed IDC & ILC	3	50 %	0	0 %	3	50 %		
	Metaplastic carcinoma	0	0 %	3	25 %	9	75 %		
	Adenoid cystic carcinoma	3	50 %	0	0 %	3	50 %		
	Total	8	13.3 %	8	13.3 %	44	73.3 %		
Nodal status	N0	4	22.2 %	3	16.7 %	11	61.1 %	10.303	0.106
	N1	1	6.3 %	3	18.8 %	12	75 %		
	N2	0	0 %	2	11.1 %	16	88.9 %		
	N3	3	37.5 %	0	0 %	5	62.5 %		
		8	13.3 %	8	13.3 %	44	73.3 %		
Tumor site	U.O.Q	3	11.1 %	3	11.1 %	21	77.8 %	17.371	0.026*
	L.O.Q	1	6.7 %	2	13.3 %	12	80 %		
	Central	4	44.4 %	0	0 %	5	55.6 %		
	U.I.Q	0	0 %	3	50 %	3	50 %		
	L.I.Q	0	0 %	0	0 %	3	100 %		
		8	13.3 %	8	13.3 %	44	73.3 %		
Tumor grade	G Ⅰ	0	0 %	3	100 %	0	0 %	22.142	0.002*
	G Ⅱ	4	22.2 %	1	5.6%	13	72.2 %		
	G Ⅲ	4	10.3%	4	10.3 %	31	79.5 %		
	Total	8	13.3 %	8	13.3 %	44	73.3 %		

and tumor side, tumor grade & nodal status with p-value (0.024, 0.024 & 0.011 respectively). There was statistically significant moderate negative correlation between E-cadherin & histological types with p-value = 0.001. There was statistically significant weak positive correlation between E-cadherin & tumor site with p-value (0.031). The other variables showed insignificant correlations with p-value (more than 0.05). Positive correlation existed between E-cadherin and age group& intra ductal component. Negative correlation existed between E-cadherin and the rest of variables. Regarding Correlation between E-Cadherin, Ki67 and different variables, there was insignificant (with P-value 0.517) negative correlation between E-cadherin & Ki-67 (with rs value -0.085) as illustrated in Table 4.

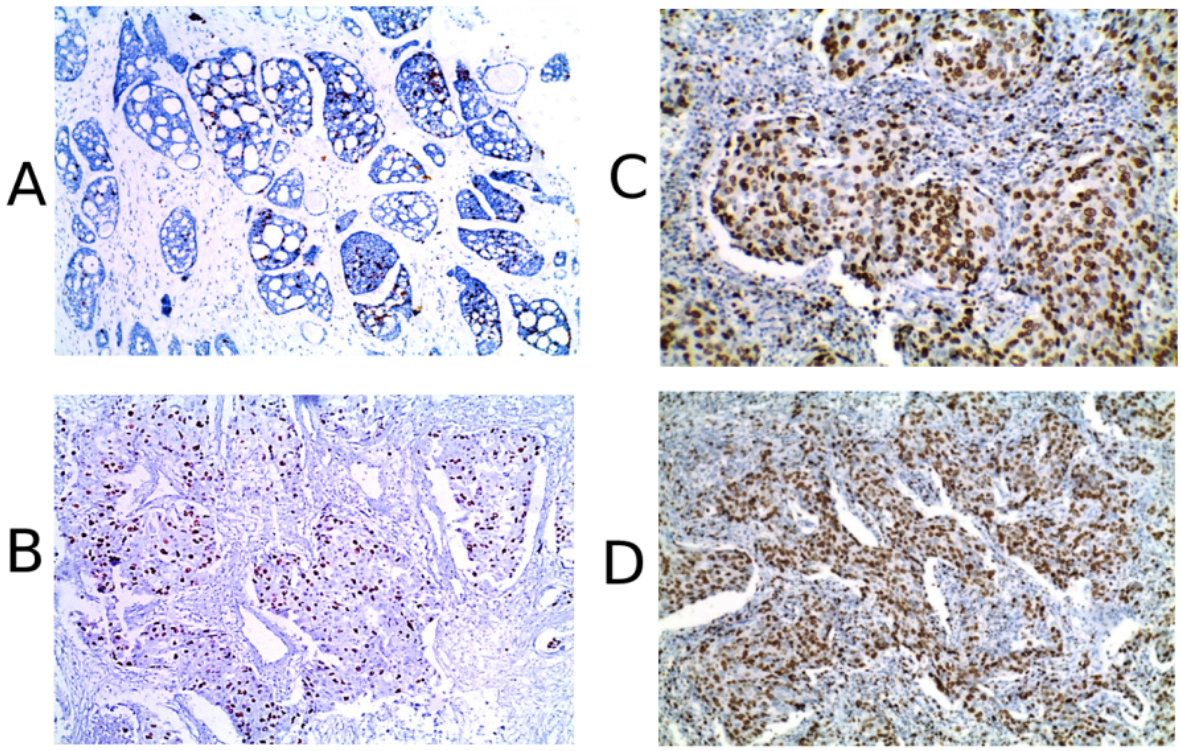

Figure 4. Immunohistochemical expression of Ki-67. (A) A case of Adenoid cystic carcinoma showing low KI67, 22% [streptavidin biotin, x100], (B) A case of Metaplastic carcinoma showing moderate Ki67 expression, 40% [streptavidin biotin, x100], (C) A case of IDC with medullary features showing high Ki67 expression, 60% [streptavidin biotin, x200], (D) A case of IDC with medullary features showing high KI-67 index, 70% [streptavidin biotin, x100].

Table 4. Correlation between E-Cadherin, Ki-67 and different variables.

Variables	Ki-67		E-Cadherin	
	r_s	P	r_s	P
Age groups	0.227	0.082	0.016	0.905
Tumor side	0.145	0.270	-0.291	0.024*
Tumor site	-0.117	0.375	0.278	0.031*
Tumor size	0.163	0.216	-0.093	0.480
Histological types	-0.251	0.053	-0.540	<0.001*
Tumor grades	0.225	0.084	-0.291	0.024*
vascular invasion	0.010	0.938	-0.168	0.200
Neural invasion	0.219	0.093	-0.084	0.522
Intra ductal carcinoma in situ	0.155	0.236	0.047	0.724
Tumor necrosis	-0.137	0.297	-0.032	0.810
Lymphocytic background	0.035	0.788	-0.051	0.700
T staging	0.170	0.194	-0.103	0.431
Nodal status	0.120	0.360	-0.325	0.011*
E-Cadherin	-0.085	0.517		
r_s: Spearman correlation *p ≤ 0.05 statistically significant				

DISCUSSION

TNBC is identified by the lack of HER2 amplification/overexpression and the absence of hormone receptor expression. Endocrine treatment and trastuzumab-targeted therapy for HER2 do not treat TNBC patients. Only cytotoxic chemotherapy is available to them as a kind of treatment. Furthermore, more than 50% of TNBCs do not respond to systemic chemotherapy, and those who do not achieve a pathologic complete response have a significant risk of local recurrence and distant metastasis, which worsens the prognosis (Shen et al., 2016). Notably, a novel panel of biomarkers was found to provide TNBC patients both prognostic and predictive insight. E cadherin expression and Ki-67 expression are two of the most encouraging indicators among them.

The current study was carried out on 60 cases of TNBC which were subjected to histopathological and immunohistochemical examination using E-cadherin and Ki-67 antibodies. The mean age of the patients was 58.8 years which was similar to previous studies (Ricciardi et al., 2015 and Adamo et al., 2017) who recorded mean age of 58.8 and 61 respectively. This slight discrepancy in the latter study may be attributed to small sample size or to racial and geographic differences. Regarding E-cadherin expression in different age groups in the present study, all cases in age group A and 91.4% of the cases in age group B were positive for E-cadherin. These results were close to (Luo et al., 2018) who reported that E-cadherin was overexpressed in TNBC with age group A and decrease with age group B.

Concerning Ki-67 expression, there was a statistical significant relation between high Ki-67 index in TNBC cases and age group (with P value 0.015) as most of cases showed high Ki-67 expression in the two age groups; 64% in age group A and 80% in age group B (Zhu et al., 2020) reported high Ki-67 expression in two age groups; 55.31% in age group A and 44.69% in age group B, this could be explained by that age <40 years was an independent risk factor for TNBC but not for the other subtypes of breast cancers. As regard tumor site, 45% of cases were from UOQ and 25% cases were from LOQ. These results were close to previous studies (Dai et al., 2018; Nabi et al., 2015) who found that most cases originating from UOQ (42%) & (50%) respectively as they compared tumor site and side between TNBC and non TNBC. But the results disagreed with previous studies (Abdollahi and Etemadi, 2016) who documented involvement of LLQ was significantly more common in TNBC. Dealing with Ki-67 index, there was a significant statistical relation between TNBC cases from lower inner quadrant and high Ki-67 index with P-value (0.026).

Most patients were determined to be T2 in terms of tumor size. This was in accordance with the research that was done by Nakagawa et al. (2011) who found that the majority of cases were T2 at presentation. This may be attributed to the rapid rate of growth of these tumors. In the current study, most of T2 cases (91.7%) showed positive E-cadherin expression, while all cases of T1 and T3 showed positive E-cadherin expression. These results were in disagreement with the results reported by (Shetty and Rao, 2019) who stated that only positive E-cadherin expression was present in 46% of T2 cases, 8% of T3 cases & 4% of T1 cases. This may be explained by that most of cases of the current study were T2 and the fact that (Shetty & Rao, 2019) excluded patients who had undergone chemotherapy prior to surgery. A previous experimental study shown that antibody-mediated E-cadherin function blockade results in a reduction in its activity and expression (Green et al., 2004).

Concerning Ki-67 expression, high Ki-67 index was noticed with increased tumor size as 83.3% of T3, 72.2% of T2 & 50% of T1 showed high Ki-67 labeling index with P value (0.061). Although these results were insignificant statistically (may be due to small sample size), they were very close to the results reported by Cheung et al. (2015) who showed statistical proportional correlation between high expression of Ki-67 and large tumor size (T2 & T3) with P value (0.047). This can be explained by that large TNBC tumors are rapidly proliferating. Regarding histological types, most instances were represented by IDC-NST. 40% of all patients included in this investigation were of this subtype. Similarly, (Ricciardi et al., 2015) reported that IDC-NST was the most common type in their study, constituting 77.7%., while, (Shetty & Rao, 2019) reported that IDC-NST represented 85.1% of cases. This little difference might be because of large scale of cases included in their study.

In the present work, all variants collected showed positive E-cadherin expression except cases of pleomorphic lobular carcinoma that showed complete loss of E-cadherin expression with significant statistical relation between expression of E-cadherin in TNBC and pleomorphic lobular subtype (P-value < 0.001). However, Shetty & Rao (2019) reported that 20% of pleomorphic lobular carcinoma (one cases of five) expressed positive E-cadherin expression with no significant statistical relation. This may be due to inappropriate antibody used or inaccurate diagnosis of this case. Concerning Ki-67, high Ki-67 labeling index

was detected in 100% of pleomorphic lobular carcinoma, 87.5% of IDC-NST, 75% of metaplastic carcinoma, 55.6% of IDC with medullary features and 50% of both mixed IDC and ILC and adenoid cystic carcinoma with significant statistical proportional relation with histological types, P-value (0.009).

In contrast, these findings were inconsistent with (Pan et al., 2017) who reported that high Ki-67 was detected in 92.3% of ductal carcinoma, 5.4% of lobular carcinoma and 2.3% of medullary carcinoma with no significant statistical relation and also different from Wang et al. (2016) who reported that high expression level of Ki- 67 in TNBC was more common in IDC (94.6%) compared with non-IDC ($p < 0.001$). A possible explanation may be that they included cases of lobular carcinoma and these are low grade types with low proliferation index. In agreement with Jing et al. (2020) the present study found that 36.7% of TNBC cases showed vascular invasion. This could be explained by the fact that TNBCs are usually metastasizing tumors.

Among cases with vascular invasion E-cadherin was immunoexpressed in 95.5% of the studied cases. This result was close to (Alaa Edin et al., 2021) who reported that 75% of cases with vascular invasion showed positive E-cadherin immunostaining. No statistically significant association between the expression of E-cadherin positively and angio-invasion was seen.

Concerning Ki-67 expression, 72.7% of cases with vascular invasion showed high Ki-67 labeling index. These results disagreed with (Ameh-Mensah et al., 2021) who reported only 18.5 % of cases with vascular invasion showed high Ki-67 labeling index, this may be due to different method of evaluation of Ki-67.

The majority of ductal cancer in situ patients (80%) had no intra duct component, while 20% of cases showed concomitant intra ductal in situ abnormalities. In contrast to Cheung et al.(2015) who reported that only (31%) of TNBC cases showed no associated in situ component.

Concerning Ki-67 expression in cases with DCIS component, (58.3%) showed high Ki-67 expression with no statistically significant correlation between both, these results disagreed with Arafah et al. (2021), who reported that only (34%) of cases with DCIS showed high Ki-67 labeling index and showed significant statistical correlation between both with P value (0.01).

This difference may be related to the amount of DCIS in the current study that ranged from 5% to 20%, but in their study the percentage of DCIS component was not specified.

Intense lymphocytic background was detected in 40% of TNBC cases. This was lower than Pistelli et al. (2014) who reported that only (16%) of TNBC cases showed lymphocytic infiltration. Regarding Ki-67 index, 70% of cases with associated lymphocytic background showed high Ki-67 labeling index with in significant statistical relation between lymphocytic background in TNBC. However, Pruneri et al. (2016) documented that 56.9% of TNBC cases with lymphocytic background showed high Ki-67 index with significant statistical relation P value (<0.0001). Regarding tumor grade, the majority of cases 65% were poorly differentiated (grade III). This was similar to previous studies (Adamo et al., 2017; Ilie et al., 2018), who stated that the majority of their TNBC cases were grade III. As most of triple negative carcinoma cases are high grade by definition.

At the current work, marked E-cadherin expression was detected in all cases of grade and in more than half of grade II cases 55.6%, while in grade III it was present in 35.9% of cases with significant statistical correlation between E-cadherin expression and tumor grade with P-value (0.024). In contrast to Luo et al. (2018) , who reported that 68.75% of grade III and only 31.25% of grade I & II showed marked E-cadherin expression without significant statistical relation However, significant statistical relation was reported between E-cadherin expression and tumor grade (P- value 0.04) (Nakagawa et al., 2011).

Dealing with Ki-67 index, 79.5% of grade III cases showed high Ki-67 labeling index, and 72.2% of cases of grade II showed high Ki-67 labeling index with significant statistical proportional relation between Ki-67 expression and tumor grade in TNBC with P-value (0.002). Similarly,

previous studies (Arafah et al., 2021; Zhu et al., 2020) reported that 98% and 83.76% of grade III showed high Ki-67 labeling index with significant statistical relation between high tumor grade & high Ki-67 labeling index (P-value <.001) and (P-value <.0001) respectively.

Axillary lymph node deposits were detected in 70% of the studied TNBC cases. Similarly, previous studies (Nabi et al., 2015) reported that (69.3%) of TNBC cases showed positive lymph node metastasis, but (Pistelli et al., 2014) detected regional lymph node metastasis in only (38.3%) of cases.

In the present study, regarding cases with positive nodal metastasis, significant statistical negative proportional correlation was found between E-cadherin expression and axillary lymph node metastasis (with P value 0.006). This was different from previous studies (Shetty and Rao, 2019) who reported that 29% of cases without nodal metastasis showed positive E-cadherin expression and 29.7% of cases with nodal metastasis showed positive E-cadherin expression. Concerning Ki-67 labeling index, insignificant statistical relation was found between nodal status in TNBC cases and Ki-67 proliferative index with P-value (0.106). In agreement with Zhu et al. (2020), who also reported insignificant statistical relation between both groups with P-value (0.1932). There was no significant correlation exist between E-cadherin expression & Ki-67 with P-value (0.517). This agreed with (Shetty & Rao, 2019) who reported that there was no significant association noted between E cadherin expression and Ki-67 expression (p value=0.977).

In breast cancer, loss of E-cadherin has been associated with increased metastasis, higher tumor grade, and larger tumor sizes as documented in previous studies and confirmed by the current study. In TNBC, downregulation of E-cadherin expression is a defining feature of the epithelial-to-mesenchymal transition and is linked to chemoresistance (Adamo et al., 2017). Ki-67 has been intensively researched as a prognostic and predictive biomarker in breast cancer. However, very few research have specifically explored its expression in TNBCs. In their study of the relationship between pre-therapeutic Ki-67 levels and the accomplishment of pathological complete response (pCR) (Arafah et al., 2021) concentrated on the Ki-67 as a potential biomarker of aggressive disease and discovered that patients with a high expression of this protein failed in achieving pCR.

CONCLUSIONS

Decreased E-cadherin expression is related to positive nodal metastasis. Also, there was a significant statistical negative relation between E-cadherin expression and pleomorphic lobular subtype. So, the expression level of E-cadherin, is of discriminative prognostic value, as its reduced expression in TNBC may be associated with tumor aggressiveness. Regarding Ki-67 expression, there was a significant statistical relation between high Ki-67 expression and age of patients, lower inner quadrant tumors and high-grade tumors. This indicates an increased proliferation of tumor cells, enhanced invasiveness, and faster growth of TNBC. E-cadherin positivity is associated with better prognosis (low TNM stage) while high Ki-67 labeling index associated with bad prognosis.

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CONFLICT OF INTEREST

The authors did not have any conflict of interest.

REFERENCES

Abdollahi, A., & Etemadi, M. (2016). Pathological Characteristics of Triple-Negative Breast Cancer at Main Referral Teaching. International Journal of Hematology-Oncology and Stem Cell Research, 5(4).

Adamo, B., Ricciardi, G. R. R., Ieni, A., Franchina, T., Fazzari, C., Sanò, M. V., Angelico, G., Michele, C., Tuccari, G., & Adamo, V. (2017). The prognostic significance of combined androgen receptor, E-Cadherin, Ki67 and CK5/6 expression in patients with triple negative breast cancer. Oncotarget, 8(44). https://doi.org/10.18632/oncotarget.20293

Alaa Edin, H. S. E., Ismail, S. A. R., Osman, G. S., Wahman, M. M., & Muhammad, E. M. S. (2021). Immunohistochemical Expression of E-cadherin in Invasive Duct Carcinoma of the Breast. SVU-International Journal of Medical Sciences, 4(1). https://doi.org/10.21608/svuijm.2021.112059

Ali, A. H., Haider, G., Ahmed, K., Hashmi, M., Kerio, P., & Irshad, R. (2020). Correlation between E-cadherin and hormone receptor status among breast cancer patients. Journal of the College of Physicians and Surgeons Pakistan, 30(10). https://doi.org/10.29271/jcpsp.2020.10.1030

Ameh-Mensah, C., Duduyemi, B. M., Bedu-Addo, K., Atta Manu, E., Opoku, F., & Titiloye, N. (2021). The Analysis of bcl-2 in Association with p53 and Ki-67 in Triple Negative Breast Cancer and Other Molecular Subtypes in Ghana. Journal of Oncology, 2021. https://doi.org/10.1155/2021/7054134

Arafah, M. A., Ouban, A., Ameer, O. Z., & Quek, K. J. (2021). KI-67 LI Expression in Triple-Negative Breast Cancer Patients and Its Significance. Breast Cancer: Basic and Clinical Research, 15. https://doi.org/10.1177/11782234211016977

Cheung, S. Y., Boey, Y. J. Y., Koh, V. C. Y., Thike, A. A., Lim, J. C. T., Iqbal, J., & Tan, P. H. (2015). Role of epithelial–mesenchymal transition markers in triple-negative breast cancer. Breast Cancer Research and Treatment, 152(3). https://doi.org/10.1007/s10549-015-3485-1

Dai, D., Jin, H., & Wang, X. (2018). Nomogram for predicting survival in triple-negative breast cancer patients with histology of infiltrating duct carcinoma: a population-based study. American Journal of Cancer Research, 8(8).

Focke, C. M., Bürger, H., van Diest, P. J., Finsterbusch, K., Gläser, D., Korsching, E., Decker, T., Anders, M., Bollmann, R., Eiting, F., Friedrich, K., Habeck, J. O., Haroske, G., Hinrichs, B., Behrens, A., Krause, U., Lang, U., Lorenzen, J., Minew, N., Woziwodski, J. (2017). Interlaboratory variability of Ki67 staining in breast cancer. European Journal of Cancer, 84. https://doi.org/10.1016/j.ejca.2017.07.041

Giuliano, A. E., Edge, S. B., & Hortobagyi, G. N. (2018). Eighth Edition of the AJCC Cancer Staging Manual: Breast Cancer. In Annals of Surgical Oncology (Vol. 25, Issue 7). https://doi.org/10.1245/s10434-018-6486-6

Green, S. K., Francia, G., Isidoro, C., & Kerbel, R. S. (2004). Antiadhesive antibodies targeting E-cadherin sensitize multicellular tumor spheroids to chemotherapy in vitro. Molecular Cancer Therapeutics, 3(2). https://doi.org/10.1158/1535-7163.149.3.2

Ilie, S. M., Bacinschi, X. E., Botnariuc, I., & Anghel, R. M. (2018). Potential clinically useful prognostic biomarkers in triple-negative breast cancer: Preliminary results of a retrospective analysis. Breast Cancer: Targets and Therapy, 10. https://doi.org/10.2147/BCTT.S175556

Iqbal, B. M., & Buch, A. (2016). Hormone receptor (ER, PR, HER2/neu) status and proliferation index marker (Ki-67) in breast cancers: Their onco-pathological correlation, shortcomings and future trends. In Medical Journal of Dr. D.Y. Patil University (Vol. 9, Issue 6). https://doi.org/10.4103/0975-2870.194180

Jing, N., Ma, M.-W., Gao, X.-S., Liu, J.-T., Gu, X.-B., Zhang, M., Zhao, B., Wang, Y., Wang, X.-L., & Jia, H.-X. (2020). Development and validation of a prognostic nomogram for patients with triple-negative breast cancer with histology of infiltrating duct carcinoma. Annals of Translational Medicine, 8(21). https://doi.org/10.21037/atm-20-413

Luo, C. W., Wu, C. C., Chang, S. J., Chang, T. M., Chen, T. Y., Chai, C. Y., Chang, C. lo, Hou, M. F., & Pan, M. R. (2018). CHD4-mediated loss of E-cadherin determines metastatic ability in triple-negative breast cancer cells. Experimental Cell Research, 363(1). https://doi.org/10.1016/j.yexcr.2017.12.032

Mohamed, D., & Abo Safia, H. (2020). Immunohistochemical study of fibroblast activation protein and α-smooth muscle actin expression and distribution in triple-negative breast cancer. International Journal of Cancer and Biomedical Research, 0(0). https://doi.org/10.21608/jcbr.2020.26388.1020

Nabi, M. G., Ahangar, A., Wahid, M. A., & Kuchay, S. (2015). Clinicopathological comparison of triple negative breast cancers with non-triple negative breast cancers in a hospital in North India. Nigerian Journal of Clinical Practice, 18(3). https://doi.org/10.4103/1119-3077.153248

Nakagawa, M., Bando, Y., Nagao, T., Morimoto, M., Takai, C., Ohnishi, T., Honda, J., Moriya, T., Izumi, K., Takahashi, M., Sasa, M., & Tangoku, A. (2011). Expression of p53, Ki-67, E-cadherin, N-cadherin and TOP2A in triple-negative breast cancer. Anticancer Research, 31(6).

Nielsen, T. O., Leung, S. C. Y., Rimm, D. L., Dodson, A., Acs, B., Badve, S., Denkert, C., Ellis, M. J., Fineberg, S., Flowers, M., Kreipe, H. H., Laenkholm, A. V., Pan, H., Penault-Llorca, F. M., Polley, M. Y., Salgado, R., Smith, I. E., Sugie, T., Bartlett, J. M. S., … Hayes, D. F. (2021). Assessment of Ki67 in Breast Cancer: Updated Recommendations from the International Ki67 in Breast Cancer Working Group. Journal of the National Cancer Institute, 113(7). https://doi.org/10.1093/jnci/djaa201

Pan, Y., Yuan, Y., Liu, G., & Wei, Y. (2017). P53 and Ki-67 as prognostic markers in triplenegative breast cancer patients. PLoS ONE, 12(2). https://doi.org/10.1371/journal.pone.0172324

Pistelli, M., Caramanti, M., Biscotti, T., Santinelli, A., Pagliacci, A., de Lisa, M., Ballatore, Z., Ridolfi, F., Maccaroni, E., Bracci, R., Berardi, R., Battelli, N.,

& Cascinu, S. (2014). Androgen receptor expression in early triple-negative breast cancer: Clinical significance and prognostic associations. Cancers, 6(3). https://doi.org/10.3390/cancers6031351

Pradhan, A., Paudyal, P., Sinha, A., & Agrawal, C. (2017). Grading, staging and Nottingham prognostic index scoring of breast carcinoma. Journal of Pathology of Nepal, 7(1). https://doi.org/10.3126/jpn.v7i1.16951

Pruneri, G., Vingiani, A., Bagnardi, V., Rotmensz, N., de Rose, A., Palazzo, A., Colleoni, A. M., Goldhirsch, A., & Viale, G. (2016). Clinical validity of tumor-infiltrating lymphocytes analysis in patients with triple-negative breast cancer. Annals of Oncology, 27(2). https://doi.org/10.1093/annonc/mdv571

Ricciardi, G. R. R., Adamo, B., Ieni, A., Licata, L., Cardia, R., Ferraro, G., Franchina, T., Tuccari, G., & Adamo, V. (2015). Androgen receptor (AR), E-Cadherin, and Ki-67 as emerging targets and novel prognostic markers in triple-negative breast cancer (TNBC) patients. PLoS ONE, 10(6). https://doi.org/10.1371/journal.pone.0128368

Shen, T., Zhang, K., Siegal, G. P., & Wei, S. (2016). Prognostic value of E-cadherin and β-catenin in triple-negative breast cancer. American Journal of Clinical Pathology, 146(5). https://doi.org/10.1093/AJCP/AQW183

Shetty, J., & Rao, C. (2019). Expression of E cadherin and Ki-67: Emerging Prognostic Markers in Triple-Negative Breast Cancer. Indian Journal of Surgical Oncology, 10(2). https://doi.org/10.1007/s13193-019-00885-x

Tan, P. H., Ellis, I., Allison, K., Brogi, E., Fox, S. B., Lakhani, S., Lazar, A. J., Morris, E. A., Sahin, A., Salgado, R., Sapino, A., Sasano, H., Schnitt, S., Sotiriou, C., van Diest, P., White, V. A., Lokuhetty, D., & Cree, I. A. (2020). The 2019 World Health Organization classification of tumours of the breast. In Histopathology (Vol. 77, Issue 2). https://doi.org/10.1111/his.14091

Wang, W., Wu, J., Zhang, P., Fei, X., Zong, Y., Chen, X., Huang, O., He, J. R., Chen, W., Li, Y., Shen, K., & Zhu, L. (2016). Prognostic and predictive value of Ki-67 in triple-negative breast cancer. Oncotarget, 7(21). https://doi.org/10.18632/oncotarget.9075

Zhu, X., Chen, L., Huang, B., Wang, Y., Ji, L., Wu, J., Di, G., Liu, G., Yu, K., Shao, Z., & Wang, Z. (2020). The prognostic and predictive potential of Ki-67 in triple-negative breast cancer. Scientific Reports, 10(1). https://doi.org/10.1038/s41598-019-57094-3