

Significance of Ubiquitin-Conjugating Enzyme (UBE2C) and P53 Expression in Molecular Subtypes of Breast Carcinoma; An Immunohistochemical Study

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Abstract:

Background: Breast cancer (BC) is the most common malignant tumor in females worldwide. It is categorized into four main molecular subtypes. Ubiquitin-conjugating enzyme (UBE2C) is essential for the ubiquitin-proteasome system which regulates checkpoints in the cell cycle. Dysregulation of ubiquitination has been associated with different types of cancer. P53 is important tumor suppressor gene. In some cancers mutant p53 proteins not only lose tumor suppressive functions, but also acquire oncogenic activity. **Aim:** This study aims at evaluation of UBE2C and P53 expression in molecular subtypes of breast cancer and assessment of their role in pathogenesis and tumor progression. **Materials and methods:** This controlled retrospective study included selected 50 cases of breast cancer mastectomy specimens. UBE2C and P53 immunostaining were performed for all cases. **Results:** UBE2C expression showed highly significant statistical association with tumor stage ($P < 0.01$) and was significantly related to molecular subtypes, tumor grade, lymphovascular invasion (LVI) and LN metastasis ($P < 0.05$). P53 expression showed highly significant association with LVI ($P < 0.01$) and was significantly related to molecular subtypes, tumor grade, LN metastasis and tumor stage ($P < 0.05$). No significant relations were found between UBE2C and P53 expressions and tumor size (pT), tumor associated with DCIS or paget's disease ($P > 0.05$). A highly significant statistical correlation was found between UBE2C and P53 expressions in molecular subtypes of cancer breast cases ($P < 0.01$). **Conclusion:** UBE2C and P53 may have a role in progression of breast cancer and may be used to develop target therapy.

Keywords: breast cancer; UBE2C; P53.

Introduction

Breast cancer (BC) is the most common female cancer and the first leading cause of global cancer incidence surpassing cancer Lung in 2020 and the fifth leading cause of cancer mortality⁽¹⁾. In Egypt, Breast cancer is the most prevalent cancer among Egyptian women representing 38.85% of total female cancer cases⁽²⁾ and the second cause of total Egyptian cancer mortality after hepatocellular carcinoma⁽³⁾ Breast cancer typically is diagnosed in middle-aged and older women with median age 63 years⁽⁴⁾. The major risk factors for breast cancer are genetic and hormonal; tumors can therefore be divided into hereditary cases associated with germline mutations and sporadic cases related to hormonal exposures with de novo mutations⁽⁵⁾

Classification of breast cancer into relevant molecular subtypes is an important aspect of therapeutic decision-making. Classical immunohistochemical markers such as ER, PR, HER2 and Ki67 play a crucial role in molecular subtyping⁽⁶⁾

Breast cancer is highly heterogenous neoplasm. Therefore, it is of great importance to delve into the pathogenesis of breast cancer and identify new molecular markers⁽⁷⁾

Ubiquitin-conjugating enzyme (UBE2C), a crucial part of the ubiquitin-conjugating enzyme complex, is involved in the ubiquitin-proteasome system. The ubiquitin-proteasome pathway is one of the main pathways of protein degradation⁽⁸⁾

Dysregulation of the ubiquitination process initiates abnormal degradation of proteins encoded by some oncogenes and tumor suppressor genes, subsequently leading to abnormal accumulation of these proteins in the body. Therefore, the ubiquitin-proteasome proteolytic (UPP) system is closely related to the occurrence and progression of cancers⁽⁹⁾

P53 is an important tumor suppressor gene that influences multiple biological

processes, including apoptosis, cell-cycle arrest, and DNA repair. Loss of p53 function, through mutations in p53 itself or in signaling pathways, is a common feature in the majority of human cancers⁽¹⁰⁾

Ubiquitin-conjugating enzyme (UBE2C) has been implicated as a candidate oncogene in cancer progression, autophagy, and drug resistance; however, its relation to P53 in molecular subtypes of breast cancer and its underlying mechanisms are not fully elucidated⁽¹¹⁾

Materials and methods:

This is a controlled retrospective study performed upon formalin-fixed, paraffin-embedded blocks of selected 50 cases of breast cancer modified radical mastectomy specimens with axillary clearance of Egyptian female patients. Six tissue blocks of apparently normal breast tissue were taken as control group. It was performed in Pathology Department and Early Cancer Detection Unit; Benha Faculty of Medicine. Cases were processed during the years January 2017 to December 2022. The study was approved by the Ethical committee of faculty of Medicine, Benha University (MD 10-4-2021).

Medical reports were reviewed and the available clinico-pathological data, including breast cancer histological type, tumor grade, DCIS, paget's disease, Lymphovascular invasion, tumor size(pT), LN metastasis, distant metastasis and immunohistochemical reports for ER, PR, HER-2 and Ki67 were documented in a database.

Histopathological study:

The slides were stained with hematoxylin and eosin. The remarkable microscopic features such as tumor grade, associated DCIS, paget's disease and lymphovascular invasion were noted.

Assessment of histopathological type of the cases was according to 2019 WHO classification of tumors of the breast¹² and

classifying them into relevant molecular subtypes based on immunohistochemical markers (ER, PR, HER-2, Ki67)^{13,14} The molecular subtypes are Luminal A (ER+/PR+/HER2-/lowKi-67); Luminal B (ER+/PR+/HER2-/+/high Ki-67); HER2-enriched (ER-/PR-/HER2+) and triple negative breast cancers (ER-/PR-/HER2-)⁽¹⁵⁾

Histologic grading was based on the Nottingham / modified Bloom & Richardson Score according to tubule formation, nuclear pleomorphism and mitotic count⁽¹⁶⁾

In addition, tumor stage was defined according to the TNM method applied by the American Joint Committee on Cancer (AJCC) and International Union for Cancer Control (UICC) (T, tumor; N, nodes; M, metastases) depending on tumor size, nodal metastases and distant metastases⁽¹⁷⁾

Immunohistochemical study:

Slides were immune stained with UBE2C antibody (diluted primary Rabbit polyclonal antibody (1:100) (**Abx Ltd, Cambridge, UK. Cat No abx302458, conc**)) and P53 antibody (the primary Rabbit ready to use monoclonal antibody (**DAKO Agilent Technologies, Inc, USA. Cat No P04637**)). Immunodetection was carried out using a standard labeled streptavidin-biotin system (**Genemed, CA 94080, USA, South San Francisco**). It was performed based on manufacturer's instructions. DAB was used as chromogen. Normal human placental tissue and high-grade serous ovarian carcinoma were used as external positive control for UBE2C and P53 respectively. Negative control was obtained by processing tissue section with omitting the primary antibody and adding Phosphate Buffered Saline (PBS) instead.

Interpretation of UBE2C immunohistochemical staining:

The positive UBE2C signal was localized

to the cytoplasm. The staining of tumor cells was brownish cytoplasmic staining with intensity scored as follows: 0 for no staining, 1 for weak, 2 for moderate and 3 for strong cytoplasmic staining. The percentage of positive cells was subdivided into four groups: 0 for less than 6%, 1 for 6–25%, 2 for 26–50%, 3 for 51–75% and 4 for more than 75%.

Multiplication of the two scores provided the final immunohistochemistry score. The eventual determination of the results was defined as follows: 0 for negative (-), 1–2 for weak positive (+), 3–4 for moderate positive (++) and ≥ 6 for strong positive (+++) ⁽¹⁸⁾

Interpretation of P53 immunohistochemical staining:

Positivity was considered as brownish nuclear staining of tumor cells with intensity scored as follows: 0 for no staining, 1 for weak, 2 for moderate and 3 for strong nuclear staining. The percentage of p53 immunoreactive cells was scored as 0 to 3+ in positive regions. Nuclear p53 expression in <10% of tumor cells was scored as negative, while $\geq 10\%$ was positive (10%- 30% +, 31%-50% ++, and >50% +++) ⁽¹⁹⁾

To compare all of the available data, an overall Histochemical Score (H-score) was assigned to each case by multiplying the intensity score by the percentage of stained cells, and a final score of 0 to 300 was given.

Two potential cutoffs to separate weak from moderate staining: at around H-scores of 15 and 50. Subgroups according to different levels of staining: negative (<15), weak positive ($\geq 15, < 50$), moderate positive ($\geq 50, < 150$) and strong positive (≥ 150) ⁽²⁰⁾

Statistical analysis

Results were analyzed using SPSS (version 22) statistical package for Microsoft windows (SPSS Inc., Chicago, IL, USA). Categorical data were expressed as numbers and percentages.

χ^2 (Chi square test), FET (Fisher's

Exact test) and Spearman`s correlation were used to assess relations between groups. P-value >0.05 was considered non-significant (NS), <0.05 significant (S), ≤ 0.01 highly significant (HS).

Results:

Clinicopathological results: (figures 1A, 1D, 2A, 2D)

The examined 50 breast cancer cases included 16 cases (32%) of luminal A, 12 cases (24%) of luminal B, 10 cases (20%) of HER2-enriched and 12 cases (24%) of triple negative subtype. The results revealed a highly significant statistical association between molecular subtypes of breast cancer and tumor grade ($P < 0.01$) and significant statistical associations with lymphovascular invasion, LN metastasis and tumor stage ($P < 0.05$). However, no significant statistical relation was found between molecular subtypes and tumor size (pT), tumor associated with DCIS or paget`s disease ($P > 0.05$).

Immunohistochemical Results:

UBE2C expression in studied cases:

Immunohistochemical results of UBE2C cytoplasmic expression in studied cases revealed 7 cases (14%) with negative cytoplasmic expression, 9 cases (18%) showing weak expression (figures 1B, 1E), 8 cases (16%) with moderate expression and 26 cases (52%) showing strong cytoplasmic UBE2C expression (figures 1C, 1F)

The relation between UBE2C expression and clinicopathological data were summarized in table (1). The results revealed a highly significant statistical association between UBE2C expression and tumor stage ($P < 0.01$) and significant statistical associations with molecular subtypes, tumor grade, lymphovascular invasion and LN metastasis ($P < 0.05$). No significant statistical relation was found between UBE2C and tumor size (pT), tumor associated with DCIS or paget`s disease ($P > 0.05$)

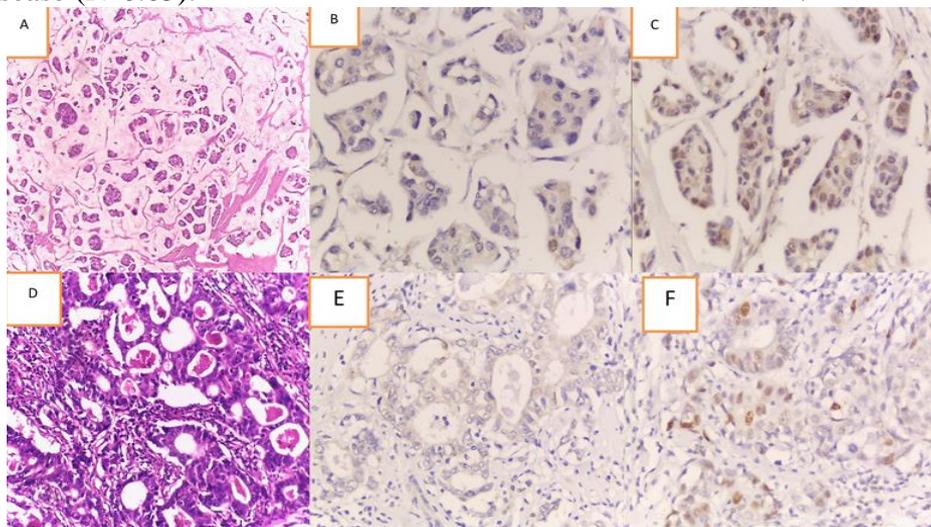


Figure (1):

A: Mucinous adenocarcinoma showing clusters and nests of tumor cells floating in pools of extracellular mucin separated by fibrous septa (H&E x100)

B: low grade breast cancer (mucinous adenocarcinoma) showing weak cytoplasmic expression of UBE2C (IHC x400)

C: low grade breast cancer (mucinous adenocarcinoma) showing weak nuclear expression of P53 (IHC x400)

D: Invasive duct carcinoma, showing ducts lined by malignant epithelial cells (H&E x200)

E: low grade breast cancer (invasive duct carcinoma) showing weak cytoplasmic expression of UBE2C (IHC x400)

F: low grade breast cancer (invasive duct carcinoma) showing weak nuclear expression of P53 (IHC x400)

Table (1): Relations of UBE2C expression with clinico-pathological and histo-pathological parameters

Clinico-pathological parameters	UBE2C expression				P-
	Negative (7 cases)	Weak (9 cases)	Moderate (8 cases)	Strong (26 cases)	
Molecular subtype					
• Luminal A	5 (31.2%)	5 (31.2%)	2 (12.5%)	4 (25%)	
• Luminal B	0 (0%)	3 (25%)	4 (33.3%)	5 (41.7%)	
• HER2-enriched	1 (10%)	1 (10%)	0 (0%)	8 (80%)	<0.05*
• Triple negative	1 (8.3%)	0 (0%)	2 (16.7%)	9 (75%)	
Tumor grade					
• Grade I	2 (50%)	1 (25%)	0 (0%)	1 (25%)	
• Grade II	3 (10.7%)	8 (28.6%)	6 (21.4%)	11 (39.3%)	<0.05*
• Grade III	2 (11.1%)	0 (0%)	2 (11.1%)	14 (77.8%)	
DCIS					
• Present	0 (0%)	4 (20%)	3 (15%)	13 (65%)	
• Absent	7 (23.3%)	5 (16.7%)	5 (16.7%)	13 (43.3%)	>0.05
Paget's disease					
• Present	0 (0%)	1 (11.1%)	1 (11.1%)	7 (77.8%)	
• Absent	7 (17.1%)	8 (19.5%)	7 (17.1%)	19 (46.3%)	>0.05
Lymphovascular invasion					
• Present	2 (8.7%)	1 (4.3%)	5 (21.7%)	15 (65.2%)	
• Absent	5 (18.5%)	8 (29.6%)	3 (11.1%)	11 (40.7%)	<0.05*
Tumor size (pT)					
❖ T1	3 (30%)	4 (40%)	0 (0%)	3 (30%)	
❖ T2	4 (18.2%)	4 (18.2%)	3 (13.6%)	11 (50%)	
❖ T3	0 (0%)	1 (9.1%)	4 (36.4%)	6 (54.5%)	>0.05
❖ T4	0 (0%)	0 (0%)	1 (14.3%)	6 (85.7%)	
LN metastasis					
❖ N0	6 (21.4%)	8 (28.6%)	3 (10.7%)	11 (39.3%)	
❖ N1	0 (0%)	1 (25%)	1 (25%)	2 (50%)	
❖ N2	1 (9.1%)	0 (0%)	4 (36.4%)	6 (54.5%)	<0.05*
❖ N3	0 (0%)	0 (0%)	0 (0%)	7 (100%)	
Tumor stage					
□ Stage I	3 (37.5%)	4 (50%)	0 (0%)	1 (12.5%)	
□ Stage II	3 (16.7%)	5 (27.8%)	4 (22.2%)	6 (33.3%)	
□ Stage III	1 (5%)	0 (0%)	4 (20%)	15 (75%)	<0.01**
□ Stage IV	0 (0%)	0 (0%)	0 (0%)	4 (100%)	

UBE2C: Ubiquitin Conjugating Enzyme E2C, DCIS: Ductal carcinoma insitu, pT= Tumor size, LN= Lymph node, *: significant, **: highly significant.

P53 expression in studied cases:

Immunohistochemical results of P53 nuclear expression in studied cases revealed 9 cases (18%) with negative P53 expression, 6 cases (12%) showing weak nuclear expression (figures 2B, 2E), 8 cases (16%) moderate and 27 cases (54%) showing strong P53 nuclear expression (figures 2C, 2F)

The relation between P53 expression and clinicopathological data were summarized in table (2). The results revealed a highly significant statistical association between P53 expression and tumor LVI (P<0.01)

and significant statistical associations with molecular subtypes, tumor grade, LN metastasis and tumor stage (P<0.05). No significant statistical relation was found between P53 and tumor size (pT), tumor associated with DCIS or paget's disease (P>0.05).

Correlation between UBE2C and P53 expression in studied cases:

There was a highly significant statistical correlations between UBE2C and P53 expressions in molecular subtypes of cancer breast cases (p<0.01) (Table 3)

Table (2): Relations of P53 expression with clinico-pathological and histo-pathological parameters

Clinico-pathological parameters	P53 expression				P-value
	Negative (9 cases)	Weak (6 cases)	Moderate (8 cases)	Strong (27 cases)	
Molecular subtype					
• Luminal A	6 (37.5%)	3 (18.8%)	3 (18.8%)	4 (25%)	<0.05*
• Luminal B	1 (8.3%)	2 (16.7%)	3 (25%)	6 (50%)	
• HER2-	0 (0%)	1 (10%)	0 (0%)	9 (90%)	
• Triple	2 (16.7%)	0 (0%)	2 (16.7%)	8 (66.7%)	
Tumor Grade					
• Grade I	2 (50%)	1 (25%)	0 (0%)	1 (25%)	<0.05*
• Grade II	6 (21.4%)	5 (17.9%)	6 (21.4%)	11 (39.3%)	
• Grade III	1 (5.6%)	0 (0%)	2 (11.1%)	15 (83.3%)	
DCIS					
• Present	1 (5%)	3 (15%)	2 (10%)	14 (70%)	>0.05
• Absent	8 (26.7%)	3 (10%)	6 (20%)	13 (43.3%)	
Paget's disease					
• Present	3 (33.3%)	0 (0%)	2 (22.2%)	4 (44.4%)	>0.05
• Absent	6 (14.6%)	6 (14.6%)	6 (14.6%)	23 (56.1%)	
Lymphovascular invasion					
• Present	2 (8.7%)	0 (0%)	3 (13%)	18 (78.3%)	<0.01**
• Absent	7 (25.9%)	6 (22.2%)	5 (18.5%)	9 (33.3%)	
Tumor size (pT)					
❖ T1	3 (30%)	3 (30%)	1 (10%)	3 (30%)	>0.05
❖ T2	3 (13.6%)	2 (9.1%)	3 (13.6%)	14 (63.6%)	
❖ T3	0 (0%)	1 (9.1%)	4 (36.4%)	6 (54.5%)	
❖ T4	3 (42.9%)	0 (0%)	0 (0%)	4 (57.1%)	
LN metastasis					
❖ N0	8 (28.6%)	6 (21.4%)	5 (17.9%)	9 (32.1%)	<0.05*
❖ N1	0 (0%)	0 (0%)	2 (50%)	2 (50%)	
❖ N2	1 (9.1%)	0 (0%)	1 (9.1%)	9 (81.8%)	
❖ N3	0 (0%)	0 (0%)	0 (0%)	7 (100%)	
Tumor stage					
□ Stage I	3 (37.5%)	3 (37.5%)	1 (12.5%)	1 (12.5%)	<0.05*
□ Stage II	3 (16.7%)	3 (16.7%)	5 (27.8%)	7 (38.9%)	
□ Stage III	3 (15%)	0 (0%)	2 (10%)	15 (75%)	
□ Stage IV	0 (0%)	0 (0%)	0 (0%)	4 (100%)	

P53: Protein 53, DCIS: Ductal carcinoma insitu, pT= Tumor size, LN= Lymph node, *: significant, **: highly significant

Table (3): Relations between UBE2C and P53 immunohistochemical expressions (spearman's correlation test)

UBE2C expression (H-score groups)	Total	P53 expression (H-score groups)								PV
		Negative		Weak		Moderate		Strong		
		NO.	%	NO.	%	NO.	%	NO.	%	
Negative	7	4	57.1%	0	0%	1	14.3%	2	28.6%	<0.01**
weak	9	1	11.1%	6	66.7%	1	11.1%	1	11.1%	
moderate	8	1	12.5%	0	0%	4	50%	3	37.5%	
strong	26	3	11.5%	0	0%	2	7.7%	21	80.8%	
Total	50	9	18%	6	12%	8	16%	27	54%	

UBE2C: Ubiquitin Conjugating Enzyme E2C, P53: Protein 53, H-Score: Histochemical Score, PV: P-Value, **: Highly Significant.

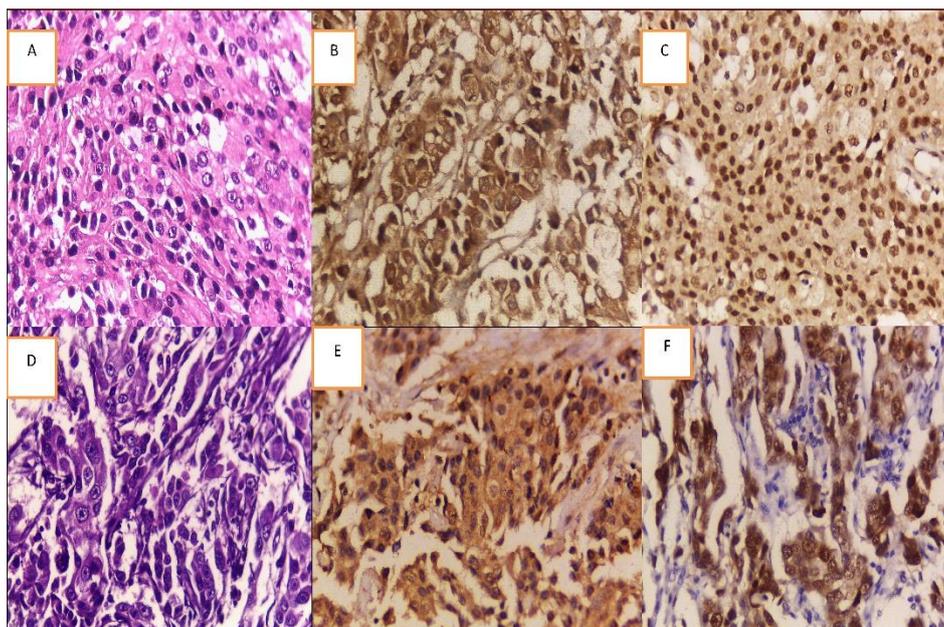


Figure (2)

A: invasive breast carcinoma of no special type (NST), showing cells with high grade nuclear atypia (H&E x400)

B: high grade breast cancer showing strong cytoplasmic expression of UBE2C (IHC x400)

C: high grade breast cancer showing strong nuclear expression of P53 (IHC x400)

D: Invasive duct carcinoma, high grade, showing sheets and nests of large pleomorphic malignant epithelial cells with marked nuclear atypia (H&E x400)

E: high grade breast cancer showing strong cytoplasmic expression of UBE2C (IHC x400)

F: high grade breast cancer showing strong nuclear expression of P53 (IHC x400)

Discussion:

Breast cancer is a highly heterogeneous neoplasm with intrinsic molecular subtypes. It is mainly classified into: luminal A, luminal B, HER2-enriched and triple negative⁽²¹⁾

Recent studies have observed that ubiquitination and deubiquitination are involved in the regulation of metabolic reprogramming in cancer cells⁽²²⁾

UBE2C is a key member of the E2 ubiquitin-binding enzyme family, encoding proteins necessary for the destruction of target proteins⁽²³⁾

Upregulation of UBE2C expression is associated with tumorigenesis and tumor progression in multiple human malignancies⁽²⁴⁾

According to immunohistochemical results of UBE2C in this study, (62.5%) of luminal A breast cancer cases showed negative and weak cytoplasmic UBE2C

expression, (58.3%) of luminal B were weak and moderate, while (80%) of HER2-enriched cases and (75%) of triple negative cases showed strong expression. There was a significant association between molecular subtypes and UBE2C expression ($p < 0.05$).

These results were in agreement with other studies who found that UBE2C expression was higher in breast cancer tissues than in adjacent tissues and positivity was higher in HER2-enriched and triple negative cases^(25, 26)

Correlating tumor grade and UBE2C expression showed a significant statistical association ($p < 0.05$). Other studies were consistent with our results and noticed progression in UBE2C immunoreactivity from normal samples to grade III breast cancer samples^(27, 28) this can be explained that UBE2C promotes tumorigenesis by activating AKT/mTOR signalling pathway and HIF-1 α and inhibiting PTEN⁽²⁹⁾

As regard carcinoma associated with DCIS or paget's disease, there were no significant statistical relations between them and UBE2C ($p > 0.05$). This was in agreement with another study⁽³⁰⁾

Concerning tumor size, p (T), 85.7% of T4 BC cases, and 54.5% of T3 showed strong UBE2C expression. Despite the positive relation, there was no significant statistical association ($p > 0.05$). The strong UBE2C expression in larger tumor size can explained that UBE2C exhibited positive associations with cyclin-related genes and cyclin B1, which play major role in cell cycle process and cell proliferation⁽²⁹⁾

In this study, significant statistical relations were found between UBE2C expression and LVI and LN metastasis ($p < 0.05$). Other studies agreed with us and found that UBE2C has major role in breast cancer invasiveness via enhancing epithelial-mesenchymal transition^(25,31,32)

Regarding tumor stage, (100%) of cases of stage IV and (75%) of stage III showed strong UBE2C expression. There was a highly significant statistical association between UBE2C expression and tumor stage ($p < 0.01$).

Many studies on UBE2C were in agreement with these results indicating the role of UBE2C in tumor progression and advanced stage. These studies were on gastrointestinal tumors, brain tumors, lung cancer and thyroid cancer⁽³³⁻³⁶⁾

In explanation, UBE2C is responsible for silencing the level of E-cadherin and enhancing the levels of N-cadherin and EGFR. This may result in the activation of cancer cell migration and invasion⁽²⁵⁾.

In this study, upregulation of UBE2C expression in breast cancer cases has been related to poor prognostic factors such as triple negative and HER2-positive subtypes, high tumor grade, positive LVI, LN metastasis and advanced tumor stage.

The transcription factor p53 is important regulator of multitude of cellular processes⁽³⁷⁾. In cancer, the tumor suppressive activities of p53 are frequently inactivated

by overexpression of its negative regulator MDM2, or mutation⁽³⁸⁾.

Mutant p53 proteins not only lose wild-type p53-dependent tumor suppressive functions, but can also acquire oncogenic activity by gain-of-function⁽³⁹⁾

According to immunohistochemical results of P53 in the current study, 25% of luminal A cases, 50% of luminal B, 90% of HER2-enriched and 66.7% of triple negative showed strong P53 expression. There was a significant statistical association between molecular subtypes of breast cancer and P53 expression ($p < 0.05$). This was in concordance with other studies who found that p53 mutation was lowest in the luminal-like subtype and highest in basal-like and HER2-amplified tumors^(40,41)

In the current study, 83.3% of grade III BC cases showed strong P53 expression. There was a significant association between P53 expression and tumor grade ($p < 0.05$). Other studies showed similar results^(42 and 43). This can be explained that mutant p53 can bind and increase the expression of chromatin-regulated genes, including methyl-transferases which enhance histone methylation and contributes to genomic instability⁽⁴⁴⁾.

Regarding breast cancer cases associated with DCIS, 70% of cases showed strong P53 expression. Despite of that, there was no significant relation ($P > 0.05$). This could be explained that DCIS associated tumors were high grade⁽⁴⁵⁾.

According to tumor size p(T), most cases of T2 (63.3%), T3 (54.5%) and T4 (57.1%) showed strong P53 expression. Despite that, there was no significant association between tumor size and P53 ($p > 0.05$). Studies were in agreement with us^(46,47), while other studies revealed a significant relation between p(T) of breast cancer and P53^(48,49). This can be explained that mutant p53 proteins activate the transcription of several genes associated with cell proliferation including c-MYC⁽⁵⁰⁾

P53 showed highly significant statistical association with LVI ($p < 0.01$) and significant associations with LN metastasis and tumor stage ($p < 0.05$). Other studies were in harmony with our findings^(40, 49, 51-53). This was parallel to studies correlating tumor stage with P53 expression in vulvar squamous cell carcinoma and ovarian serous carcinoma indicating the role of P53 in cancer progression^(54, 55)

In explanation, promotion of cancer invasion and metastasis was a well-known gain of function activity of mutant p53⁽⁵⁶⁾. Mutp53 upregulates ZEB1 to promote EMT and cancer cell invasion⁽⁵³⁾

In this study, increased expression of P53 in BC cases was related to triple negative and HER2 positive subtypes, high tumor grade, positive LVI, LN metastasis and advanced tumor stage. So according to these results, mutant P53 promotes malignant biological behavior in breast cancer.

There were highly significant statistical correlations in our study between UBE2C and P53 expressions regarding H-score, intensity and percentage of positive tumor cells in molecular subtypes of cancer breast ($p < 0.01$).

In addition, both markers showed the same significant relations with clinicopathological parameters in BC cases including, higher expressions in triple negative and HER2-positive subtypes, higher tumor grade, positive LVI, positive LN metastasis and advanced tumor stage indicating the harmony between these markers in breast cancer progression.

These results were consistent with a study which stated that, mutant p53 increased expression of UBE2C leading to impaired spindle assembly checkpoint by facilitating premature anaphase causing accelerated growth and enhanced chemoresistance in cancer cells⁽⁵⁷⁾

In agreement, studies revealed that expression of UBE2C was positively correlated with P53 expression in breast cancer^(18, 25, 58)

Another study showed that association between UBE2C and p53 is described in many tumors where UBE2C-induced p53 degradation promotes migration and invasion⁽³⁴⁾

In non-small cell lung cancer, Silencing of UBE2C induced cell apoptosis and regulated downstream genes including P53⁽⁵⁹⁾.

A study performed on endometrial cancer revealed that *UBE2C* promotes EMT in cancer cells. Estrogen modulates the expression of *UBE2C*, which in turn downregulates p53 protein expression, and leads to the promotion of cell migration and EMT⁽¹¹⁾

In HCC, PRIM1 may have role in tumor progression by increasing activity of PI3K/AKT/mTOR signalling. PRIM1 causes ubiquitination and degradation of P53 by upregulating UBE2C⁽⁶⁰⁾

In brain tumors, UBE2C silencing induces autophagy, inhibits cell viability and promotes the activation of p53⁽³⁴⁾.

Our study revealed that upregulation of UBE2C expression was positively correlated with P53 expression in breast cancer, and both were related to cancer progression and aggressive tumor characteristics.

Conclusion:

UBE2C and P53 may have a role in progression of breast cancer and may be used as prognostic markers for molecular subtypes to develop target therapy for breast cancer treatment.

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