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

**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–proteosome 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 breast**12**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) **(Abbexa 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 ≥ 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 (≥ 15,<50), moderate positive (≥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. X2 (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 revealed7 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).

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

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Clinico-pathological parameters** | | | **UBE2C expression** | | | | **P-value** |
| **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%) | **<0.05\*** |
|  | Luminal B | | 0 (0%) | 3 (25%) | 4 (33.3%) | 5 (41.7%) |
|  | HER2-enriched | | 1 (10%) | 1 (10%) | 0 (0%) | 8 (80%) |
|  | Triple negative | | 1 (8.3%) | 0 (0%) | 2 (16.7%) | 9 (75%) |
| **Tumor grade** | | | | | |
|  | Grade I | | 2 (50%) | 1 (25%) | 0 (0%) | 1 (25%) | **<0.05\*** |
|  | Grade II | | 3 (10.7%) | 8 (28.6%) | 6 (21.4%) | 11 (39.3%) |
| *  | Grade III | | 2 (11.1%) | 0 (0%) | 2 (11.1%) | 14 (77.8%) |
| **DCIS** |  | | | | |
|  | Present | | 0 (0%) | 4 (20%) | 3 (15%) | 13 (65%) | **>0.05** |
|  | Absent | | 7 (23.3%) | 5 (16.7%) | 5 (16.7%) | 13 (43.3%) |
| **Paget`** | **`s disease** | | | | |
|  | Present | | 0 (0%) | 1 (11.1%) | 1 (11.1%) | 7 (77.8%) | **>0.05** |
|  | Absent | | 7 (17.1%) | 8 (19.5%) | 7 (17.1%) | 19 (46.3%) |
| **Lymphovascular invasion** | | | | | |
|  | | | | | | |  |
| * Present | | | 2 (8.7%) | 1 (4.3%) | 5 (21.7%) | 15 (65.2%) | **<0.05\*** |
| * Absent | | | 5 (18.5%) | 8 (29.6%) | 3 (11.1%) | 11 (40.7%) |
| **Tumor size (pT)** | | | | | |
| * T1 | | | 3 (30%) | 4 (40%) | 0 (0%) | 3 (30%) | **>0.05** |
| * T2 | | | 4 (18.2%) | 4 (18.2%) | 3 (13.6%) | 11 (50%) |
| * T3 | | | 0 (0%) | 1 (9.1%) | 4 (36.4%) | 6 (54.5%) |
| * 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%) | **<0.05\*** |
| * N1 | | | 0 (0%) | 1 (25%) | 1 (25%) | 2 (50%) |
| * N2 | | | 1 (9.1%) | 0 (0%) | 4 (36.4%) | 6 (54.5%) |
| * N3 | | | 0 (0%) | 0 (0%) | 0 (0%) | 7 (100%) |
| **Tumor stage** | | | | | |
|  | | Stage I | 3 (37.5%) | 4 (50%) | 0 (0%) | 1 (12.5%) | **<0.01\*\*** |
|  | | Stage II | 3 (16.7%) | 5 (27.8%) | 4 (22.2%) | 6 (33.3%) |
|  | | Stage III | 1 (5%) | 0 (0%) | 4 (20%) | 15 (75%) |
|  | | Stage IV | 0 (0%) | 0 (0%) | 1. (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 revealed9 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).

**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-enriched | | | 0 (0%) | 1 (10%) | 0 (0%) | 9 (90%) |
|  | Triple negative | | | 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%) | 1. (0%) | 0 (0%) | 4 (100%) |

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

**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)**

**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)**

**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 (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.

**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 studieswho 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 and 26)**

Correlating tumor grade and UBE2C expression showed a significant statistical association (p<0.05). Another studies were consistent with our results and noticed progression in UBE2C immunoreactivity from normal samples to grade III breast cancer samples (**27and28)** this can be explained that UBE2C promotes tumorigenesis by activating AKT/mTOR signalling pathway and HIF-1α 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). Another studies agreed with us and found that UBE2C has major role in breast cancer invasiveness via enhancing epithelial-mesenchymal transition (**25,31and 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, 34, 35 and 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 and 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 (**46and 47)**, while other studiesrevealed a significant relation between p(T) of breast cancer and P53 (**48 and 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, 52 and53)** .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 and 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 behaviour in breast cancer.

There was 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, studiesrevealed that expression of UBE2C was positively correlated with P53 expression in breast cancer (**18, 25 and 58)**

Another studyshowed 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.

**References:**

1. **Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A,** **et al**. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2021 May;71(3):209-49.
2. **Mohamed SK.** Awareness and knowledge toward breast cancer and breast self-examination: A cross-sectional descriptive study among undergraduate female students at Cairo University, Egypt. The Malaysian Journal of Nursing (MJN). 2021 Jan 2;12(3):111-9.
3. **Azim HA, Elghazawy H, Ghazy RM, Abdelaziz AH, Abdelsalam M, Elzorkany A,** **et al**. Clinicopathologic Features of Breast Cancer in Egypt—Contemporary Profile and Future Needs: A Systematic Review and Meta-Analysis. JCO Global Oncology. 2023 Mar;9:e2200387.
4. **Giaquinto AN, Sung H, Miller KD, Kramer JL, Newman LA, Minihan A,** **et al**. Breast cancer statistics, 2022. CA: a cancer journal for clinicians. 2022 Nov;72(6):524-41.
5. **Kashyap D, Pal D, Sharma R, Garg VK, Goel N, Koundal D,** **et al**. Global increase in breast cancer incidence: risk factors and preventive measures. BioMed research international. 2022; 2022: 9605439.
6. **Turner KM, Yeo SK, Holm TM, Shaughnessy E and Guan JL**. Heterogeneity within molecular subtypes of breast cancer. American Journal of Physiology-Cell Physiology. 2021 Aug 1;321(2):C343-54.
7. **Guo L, Kong D, Liu J, Zhan L, Luo L, Zheng W, Zheng Q, et al**. Breast cancer heterogeneity and its implication in personalized precision therapy. Experimental hematology & oncology. 2023 Jan 9;12(1):3.
8. **Liu PF, Chen CF, Shu CW, Chang HM, Lee CH, Liou HH,** **et al**. UBE2C is a potential biomarker for tumorigenesis and prognosis in tongue squamous cell carcinoma. Diagnostics. 2020 Sep 4;10(9):674.
9. **Du X, Song H, Shen N, Hua R and Yang G.** The molecular basis of ubiquitin-conjugating enzymes (E2s) as a potential target for cancer therapy. International Journal of Molecular Sciences. 2021 Mar 26;22(7):3440.
10. **Sammons MA, Nguyen TA, McDade SS and Fischer M.** Tumor suppressor p53: from engaging DNA to target gene regulation. Nucleic acids research. 2020 Sep 18;48(16):8848-69.
11. **Liu Y, Zhao R, Chi S, Zhang W, Xiao C, Zhou X,** **et al**. UBE2C is upregulated by estrogen and promotes epithelial–mesenchymal transition via p53 in endometrial cancer. Molecular Cancer Research. 2020 Feb 1;18(2):204-15.
12. **Tan PH, Ellis I, Allison K, Brogi E, Fox SB, Lakhani S,** **et al**. The 2019 WHO classification of tumours of the breast. Histopathology. 2020;77(2).
13. **Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H,** **et al**. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proceedings of the National Academy of Sciences. 2001 Sep 11;98(19):10869-74.
14. **Prat A and Perou CM.** Deconstructing the molecular portraits of breast cancer. Molecular oncology. 2011 Feb 1;5(1):5-23.
15. **Al-Thoubaity FK.** Molecular classification of breast cancer: A retrospective cohort study. Annals of medicine and surgery. 2020 Jan 1;49:44-8.
16. **Kerin EP, Davey MG, McLaughlin RP, Sweeney KJ, Barry MK, Malone CM,** **et al**. Comparison of the Nottingham Prognostic Index and OncotypeDX© recurrence score in predicting outcome in estrogen receptor positive breast cancer. The Breast. 2022 Dec 1;66:227-35.
17. **Zhu H and Doğan BE.** American Joint committee on cancer’s staging system for breast cancer: summary for clinicians. European journal of breast health. 2021 Jul;17(3):234.
18. **Mo CH, Gao L, Zhu XF, Wei KL, Zeng JJ, Chen G,** **et al**. The clinicopathological significance of UBE2C in breast cancer: a study based on immunohistochemistry, microarray and RNA-sequencing data. Cancer cell international. 2017 Dec;17:1-7.
19. **Yousif WG and Al Saadawi AR.** Immunohistochemical Expression of P53 in Urothelial Bladder Lesions in a Sample of Patients. IRAQI POSTGRADUATE MEDICAL JOURNAL. 2023;22(3).
20. **Bankhead P, Fernández JA, McArt DG, Boyle DP, Li G, Loughrey MB,** **et al**. Integrated tumor identification and automated scoring minimizes pathologist involvement and provides new insights to key biomarkers in breast cancer. Laboratory investigation. 2018 Jan 1;98(1):15-26
21. **Orrantia-Borunda, E., Anchondo-Nuñez, P., Acuña-Aguilar, L. E., Gómez-Valles, F. O., and Ramírez-Valdespino, C. A.** Subtypes of breast cancer. Breast Cancer [Internet] **2022**.
22. **Dewson G, Eichhorn PJ and Komander D.** Deubiquitinases in cancer. Nature Reviews Cancer. 2023 Dec;23(12):842-62.
23. **Huang R, Guo L, Chen C, Xiang Y, Li G, Zheng J,** **et al**. System analysis identifies UBE2C as a novel oncogene target for adrenocortical carcinoma. Plos one. 2023 Aug 3;18(8):e0289418.
24. **Lu ZN, Song J, Sun TH and Sun G.** UBE2C affects breast cancer proliferation through the AKT/mTOR signaling pathway. Chinese medical journal. 2021 Oct 20;134(20):2465-74.
25. **Kariri Y, Toss MS, Alsaleem M, Elsharawy KA, Joseph C, Mongan NP,** **et al**. Ubiquitin-conjugating enzyme 2C (UBE2C) is a poor prognostic biomarker in invasive breast cancer. Breast cancer research and treatment. 2022 Apr;192(3):529-39.
26. **Shen J, Yan H, Yang C, Lin H, Li F and Zhou J.** Validation of a disease-free survival prediction model using UBE2C and clinical indicators in breast cancer patients. Breast Cancer: Targets and Therapy. 2023 Dec 31:295-310.
27. **Yu D, Liu S, Chen Y and Yang L.** Integrative bioinformatics analysis reveals CHEK1 and UBE2C as luminal a breast cancer subtype biomarkers. Frontiers in Genetics. 2022 Jul 12;13:944259.
28. **Guo Y, Chen X, Zhang X and Hu X.** UBE2S and UBE2C confer a poor prognosis to breast cancer via downregulation of Numb. Frontiers in Oncology. 2023 Feb 14;13:992233.
29. **Lu ZN, Song J, Sun TH and Sun G.** UBE2C affects breast cancer proliferation through the AKT/mTOR signaling pathway. Chinese medical journal. 2021 Oct 20;134(20):2465-74.
30. **Schandiz H, Park D, Kaiser YL, Lyngra M, Talleraas IS, Geisler J,** **et al**. Subtypes of high-grade breast ductal carcinoma in situ (DCIS): incidence and potential clinical impact. Breast Cancer Research and Treatment. 2023 Sep;201(2):329-38.
31. **Kariri YA, Aleskandarany MA, Joseph C, Kurozumi S, Mohammed OJ, Toss MS,** **et al**. Molecular complexity of lymphovascular invasion: the role of cell migration in breast cancer as a prototype. Pathobiology. 2020 Sep 15;87(4):218-31.
32. **Wang C, Pan YH, Shan M, Xu M, Bao JL and Zhao LM.** Knockdown of UbcH10 enhances the chemosensitivity of dual drug resistant breast cancer cells to epirubicin and docetaxel. International Journal of Molecular Sciences. 2015 Mar 2;16(3):4698-712.
33. **Guo C, Guo X, Wei Z, Wang Q and Zhang H.** Role of Ubiquitin-Conjugating Enzyme UBE2C in Gastrointestinal Cancers. Proceedings of Anticancer Research. 2021 Sep 30;5(5):64-72.
34. **Domentean S, Paisana E, Cascão R and Faria CC.** Role of UBE2C in Brain Cancer Invasion and Dissemination. International Journal of Molecular Sciences. 2023 Oct 31;24(21):15792.
35. **Cai D, Tian F, Wu M, Tu J and Wang Y.** UBE2C is a diagnosis and therapeutic biomarker involved in immune infiltration of cancers including lung adenocarcinoma. Journal of Cancer. 2024;15(6):1701.
36. **Li J, Li Z and Zhao P.** Diagnosis and prognosis of thyroid cancer by immune-related genes. American Journal of Clinical Oncology. 2024 Jan 1;47(1):1-0.
37. **Blagih J, Buck MD and Vousden KH.** p53, cancer and the immune response. Journal of cell science. 2020 Mar 1;133(5):jcs237453.
38. **Hassin O and Oren M.** Drugging p53 in cancer: one protein, many targets. Nature Reviews Drug Discovery. 2023 Feb;22(2):127-44.
39. **Zhang C, Liu J, Xu D, Zhang T, Hu W and Feng Z.** Gain-of-function mutant p53 in cancer progression and therapy. Journal of molecular cell biology. 2020 Sep 1;12(9):674-87.
40. **Dajti G, Serra M, Cisternino G, Ceccarelli C, Pellegrini A, Melina M,** **et al**. Prognostic Role of p53 Immunohistochemical Status in invasive Breast Cancer. A Retrospective Review of 1387 Cases With Luminal-Like/Her2 Negative Breast Tumors. The Oncologist. 2024 May 1;29(5):384-91.
41. **Dibra D, Moyer SM, El-Naggar AK, Qi Y, Su X and Lozano G.** Triple-negative breast tumors are dependent on mutant p53 for growth and survival. Proceedings of the National Academy of Sciences. 2023 Aug 22;120(34):e2308807120.
42. **Khadim MT, Ali SS, Wajid MI, Bajwa AA and Anjum S.** Immunohistochemical expression of P53 in invasive ductal carcinoma of breast in pakistani women. Pakistan Armed Forces Medical Journal. 2021 Jan 27;71(Suppl-1):S142-46.
43. **Steffens Reinhardt L, Groen K, Xavier A and Avery-Kiejda KA.** p53 Dysregulation in Breast Cancer: Insights on Mutations in the TP53 Network and p53 Isoform Expression. International Journal of Molecular Sciences. 2023 Jun 13;24(12):10078.
44. **Stein Y, Rotter V and Aloni-Grinstein R.** Gain-of-function mutant p53: all the roads lead to tumorigenesis. International journal of molecular sciences. 2019 Dec 8;20(24):6197.
45. **Morrissey RL, Thompson AM and Lozano G.** Is loss of p53 a driver of ductal carcinoma in situ progression?. British journal of cancer. 2022 Nov 9;127(10):1744-54.
46. **Arora K, Khandelwal R and Pant H.** Evaluation of p53 in breast cancer and its correlation with various histological prognostic factors. Indian J Pathol Oncol. 2020;7(3):392-8.
47. **Karakaya, Y. A., and Yılmaz, S.** CORRELATION OF GATA-3, E-CADHERIN, P53 AND KI-67 EXPRESSION WITH HISTOLOGICAL TYPE/MOLECULAR SUBTYPE AND CLINICOPATHOLOGICAL PARAMETERS IN BREAST CANCER. *International Journal of Health Services Research and Policy 2021*, *6*(1), 105-116.
48. **Li Y, Zhang X, Qiu J, Pang T, Huang L and Zeng Q.** Comparisons of p53, KI67 and BRCA1 expressions in patients with different molecular subtypes of breast cancer and their relationships with pathology and prognosis. J buon. 2019 Nov 1;24(6):2361-8.
49. **Jindal B, Mohan A, Ansari V and Sharma VK.** Role of p53 as a prognostic marker in breast carcinoma and its correlation with tumor size, tumor grade and lymph node metastasis. Indian Journal of Pathology and Oncology. 2020;7(3):378-83.
50. **Grzes M, Oron M, Staszczak Z, Jaiswar A, Nowak-Niezgoda M and Walerych D.** A driver never works alone—interplay networks of mutant p53, MYC, RAS, and other universal oncogenic drivers in human cancer. Cancers. 2020 Jun 11;12(6):1532.
51. **Nishimura R, Osako T, Okumura Y, Nakano M, Ohtsuka H, Fujisue M,** **et al**. An evaluation of lymphovascular invasion in relation to biology and prognosis according to subtypes in invasive breast cancer. Oncology Letters. 2022 Aug 1;24(2):1-8.
52. **Sharma M, Khanna M, Manjari M, Madan M, Singh T and Garg T.** Immunohistochemical characteristics of Breast Cancer patients with the comparative study of BRCA1, ER, PR, BCL2, P53 and Ki-67 immunohistochemical markers: A population based study. Ann Pathol Lab Med. 2016;3(6):490–4
53. **Parfenyev S, Singh A, Fedorova O, Daks A, Kulshreshtha R and Barlev NA.** Interplay between p53 and non-coding RNAs in the regulation of EMT in breast cancer. Cell death & disease. 2021 Jan 4;12(1):17.
54. **Carreras‐Dieguez N, Saco A, Del Pino M, Marimon L, López del Campo R, Manzotti C,** **et al**. Human papillomavirus and p53 status define three types of vulvar squamous cell carcinomas with distinct clinical, pathological, and prognostic features. Histopathology. 2023 Jul;83(1):17-30.
55. **Bates M, Mullen D, Lee E, Costigan D, Heron EA, Kernan N,** **et al**. P53 and TLR4 expression are prognostic markers informing progression free survival of advanced stage high grade serous ovarian cancer. Pathology-Research and Practice. 2024 Jan 1;253:155020.
56. **Roszkowska KA, Gizinski S, Sady M, Gajewski Z and Olszewski MB.** Gain-of-function mutations in p53 in cancer invasiveness and metastasis. International journal of molecular sciences. 2020 Feb 17;21(4):1334.
57. **Bajaj S, Alam SK, Roy KS, Datta A, Nath S and Roychoudhury S.** E2 ubiquitin-conjugating enzyme, UBE2C gene, is reciprocally regulated by wild-type and gain-of-function mutant p53. Journal of Biological Chemistry. 2016 Jul 1;291(27):14231-47.
58. **Dastsooz H, Cereda M, Donna D and Oliviero S.** A comprehensive bioinformatics analysis of UBE2C in cancers. International journal of molecular sciences. 2019 May 7;20(9):2228.
59. **Zhang Z, Liu P, Wang J, Gong T, Zhang F, Ma J,** **et al**. Ubiquitin-conjugating enzyme E2C regulates apoptosis-dependent tumor progression of non-small cell lung cancer via ERK pathway. Medical Oncology. 2015 May;32:1-7.
60. **Zhu M, Wu M, Bian S, Song Q, Xiao M, Huang H,** **et al**. DNA primase subunit 1 deteriorated progression of hepatocellular carcinoma by activating AKT/mTOR signaling and UBE2C-mediated P53 ubiquitination. Cell & Bioscience. 2021 Dec;11:1-9.