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# Investigation of telomerase activity and apoptosis on invasive ductal carcinoma of the breast using immunohistochemical and Western blot methods

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**Abstract. – OBJECTIVE: Invasive ductal carcinoma (IDC) comprises the largest group of breast cancers. This study aimed to investigate telomerase activity and apoptosis using immunohistochemical and Western blot methods.**

**PATIENTS AND METHODS: In total, 75 cases that had been diagnosed as IDC and 20 cases that had undergone a freezing procedure were included. The histological sections were stained with Bax, Bcl-2, hTERT and BNIP3. The ages of the patients, as well as their hormonal status and tumour sizes and grades were evaluated, as well as the staining characteristics of the antibodies in question.**

**RESULTS: A decrease in Bcl-2 positivity and an increase in Bax positivity were found immunohistochemically with increasing tumour grades. The data obtained by western blot method showed that Bcl-2 was highest in grade 1 tumours although these results were not statistically significant. The relationship between estrogen and progesterone receptor positivity and Bcl-2 was statistically significant, suggesting there is hormonal control through apoptosis. BNIP3 was found to be decreased with increasing tumour grades. Similarly, BNIP3 was found to be having the lowest value in grade 3 tumours by western blot method. Furthermore, hTERT was found to be increased with increasing tumour grades. In the western blot method, hTERT increased nearly four-fold compared to the control. In addition, hTERT, which was seen in very high levels in tumours, may be a helpful cancer marker. Both hTERT and BNIP3 are important markers that can provide information about prognosis.**

**CONCLUSIONS: Big improvements can be achieved in tumour progression control with new treatment modalities that stop telomerase activity and hypoxic cell death.**

*Key Words:*
Breast, Carcinoma, Apoptosis, Telomerase, Western blot, Immunohistochemistry.

## Introduction

The most common mechanism of cell death is apoptosis, i.e. programmed cell death. Many apoptotic stimulations cause cell death by effecting the Bcl-2 gene family[1]. The Bcl-2 gene family plays a key role in the regulation of apoptosis, and it shows impaired functioning in the majority of tumours, including breast cancer[2,3]. This gene family consists of both suppressor and stimulating apoptosis[1]. The Bcl-2/E1B 19 kD interacting protein (BNIP-3) is a member of the eBcl-2 family, and it shows an increase under hypoxic conditions[4,5]. In some studies, BNIP3 was significantly higher in the cytoplasms and nuclei of neoplastic cells compared to normal breast tissue in ductal carcinomas in situ and invasive breast carcinomas, This shows that BNIP-3 plays an important role in the neoplastic progression of breast cancer. Telomeres are composed of repetitive chains of specific DNA found in the ends of linear chromosomes of eukaryotic organisms[6]. Telomerase is a reverse transcriptase enzyme ca-

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pable of extending repeated nucleotide sequences (TTAGGG) at the ends of the chromosomes that cause tumours[7-9]. For tumour cells, one of the escape mechanisms from apoptosis is to continue telomerase activity. In these studies, hTERT was found to be higher in malign tumours and cancer cells when compared to normal tissues; in breast cancer, a strong relationship between the release of hTERT and telomerase activity has been detected as well[8,1].

## Patients and Methods

This study was carried out with breast biopsy and mastectomy materials received from 75 patients diagnosed with invasive ductal carcinoma (IDC). Each diagnosis was confirmed by slides that were examined by light microscopy. The histological grade, tumour size, tumour differentiation and nuclear grade were assessed separately, according to the modified Bloom-Richardson grading system[11]. Grouping was done according to tumour size (≤ 2 cm, 2-5 cm, > 5 cm)[1] and according to age (≤ 50 years and > 50 years)[12].

### Immunohistochemistry

Tumor blocks in specimens were received from 75 patients. Bax (Thermoscientific, monoclonal mouse-antibody to human Bax Ab-1, 1 ml, clone 2D2, 1/100 Fremont CA USA), Bcl-2 (Thermoscientific, BCL-2alpha Ab-1, 1/100, Fremont, CA, USA), hTERT (Abcam, rabbitmonoclonal [Y182] totelomerase, 1/75, Cambridge, UK) and BNIP3 (Abcam, rabbitpolyclonalto BNIP3L, 1/100, Cambridge, UK) were processed in automated staining equipment (Ventana Medical System, SN: 712299, REF: 750-700, AZ, USA). Hodgkin's lymphoma was used for Bax, tonsillar tissue was used for Bcl-2 and adenocarcinoma of the lung was used as a control for hTERT. Estrogen receptor (ER), progesterone receptor (PR) and c-erbB2 receptor staining were correlated with the previous report, in order to re-evaluate and confirm.

### Evaluation of Immunoreactivity

Bax, Bcl-2, hTERT and BNIP3 stained with immunohistochemical methods were examined and evaluated in terms of the staining intensities in the tumour cells. Staining intensity was considered as weak, moderate or strong. For Bax, Bcl-2, hTERT and BNIP3, poor prevalence or a prevalence of less than 10% were considered negative staining[13-15]. For Bax and Bcl-2, cytoplasmic staining was considered positive. Lymphocytes were evaluated as internal controls. For hTERT and BNIP3, both cytoplasmic and nuclear stainings were considered positive[5,14]. No immunohistochemical staining scores were made for ER and PR. Furthermore, nuclear staining of the tumour tissue of more than 10% was considered positive, while less than 10% was considered negative staining. While evaluating the c-ErbB2 expression, it was scored in four separate groups[16] as follows: Score 0: No staining in tumour cell or less than 10% cell membrane staining. Score 1: More than 10% tumour cell membranous staining or partial-light staining of the cell membrane. Score 2: More than 10% tumour cell membranous staining or light and moderately stained. Score 3: More than 10% tumour cell membranous staining totally and strongly stained. Scores 2 and 3 were considered positive while scores 0 and 1 were considered negative staining.

### Western Blot Method

For western blot, we considered six frozen IDCs and one normal breast tissue. Specimens were kept in a refrigerator at –80°C. After homogenisation of the tissues, electrophoresis of the proteins was completed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). This electrophoresis was followed by four steps, as given below. The transfer of proteins (blotting) from the gel to the nitrocellulose membrane (Schleicher and Schuell, Inc., USA): after completing SDS-PAGE, a polyacrylamide gel was to be blotted. In order to transfer to the nitrocellulose membrane with the help of the polyacrylamide gel, both the surfaces are brought together in such a way that there is no gap between the surfaces of the polyacrylamide gel and the nitrocellulose membrane. They were wrapped in a filter paper, and this system was kept in a blotting apparatus and then saturated with a buffer solution. An electric current of 150 A was applied for 60 min to the devices, which were placed in a cooled tank filled with a buffer solution. The transfer of proteins was achieved in this way. Coating of the protein-binding regions (blocking) on the nitrocellulose membrane with unrelated proteins in order to prevent non-specific reactions: after the blotting process, petri dishes were washed three times for 5 min on a shaker with the buffer solution [$NaH_2PO_4.2H_2O$ (0,025 M), $Na_2HPO_4.12H_2O$ (0,075 M), NaCl (1+, 45 M)] from which they were taken. The non-specif-

ic bindings were blocked with 1% fresh bovine serum albumin (BSA) in a 100 mMNaCl, 20 mM $Na_2HPO_4$, 20 mM $NaH_2PO_4$ (pH: 7.2) buffer solution by incubating for 90 min at 37°C. Reaction with specific antibodies: as the primary antibodies, the Bax, Bcl-2, hTERT and BNIP3 antibodies were used, and 0.05% primary antibodies were used after mixing it with a 1:1000 mixture of Tween-20 and a buffer solution. Nitrocellulose membranes along with the Bax, Bcl-2, hTERT and BNIP3 antibodies were kept at 4°C in incubation throughout the night. Later on, the nitrocellulose membranes were washed five times for 5 min in a buffer solution.

For band appearances, a solution of diaminobenzidine (DAB) mixed with 1M Tris (pH: 7.4) buffer in a ratio of 0.03-0.05 was used. The bands over the nitrocellulose membrane became visible after a short time. Then, the nitrocellulose membranes were thoroughly washed. After drying thoroughly, the nitrocellulose membranes were taken for the analysis of the relative densities of the bands. The relative densities of the bands were analysed using an Image Analyses System (Image J; The National Institute of Health, Bethesda, MD, USA) software programme.

### Statistical Analysis

The study findings were analyzed using the SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA) 12.0 programme. For a qualitative data comparison, the chi-square test and Fisher's exact chi-square test were used. The statistical significance level was accepted as 0.05.

## Results

In the study, 75 patients diagnosed with IDC were included, of which 17 were evaluated as grade I (22.67%), 48 as grade II (64%) and 10 as grade III (13.33%) (Figure 1). The age range of the study was 29-80 years and the mean age was 52.8 years. In all, 37 patients were ≤ 50 years and 38 patients were over the age of 50. The diame-

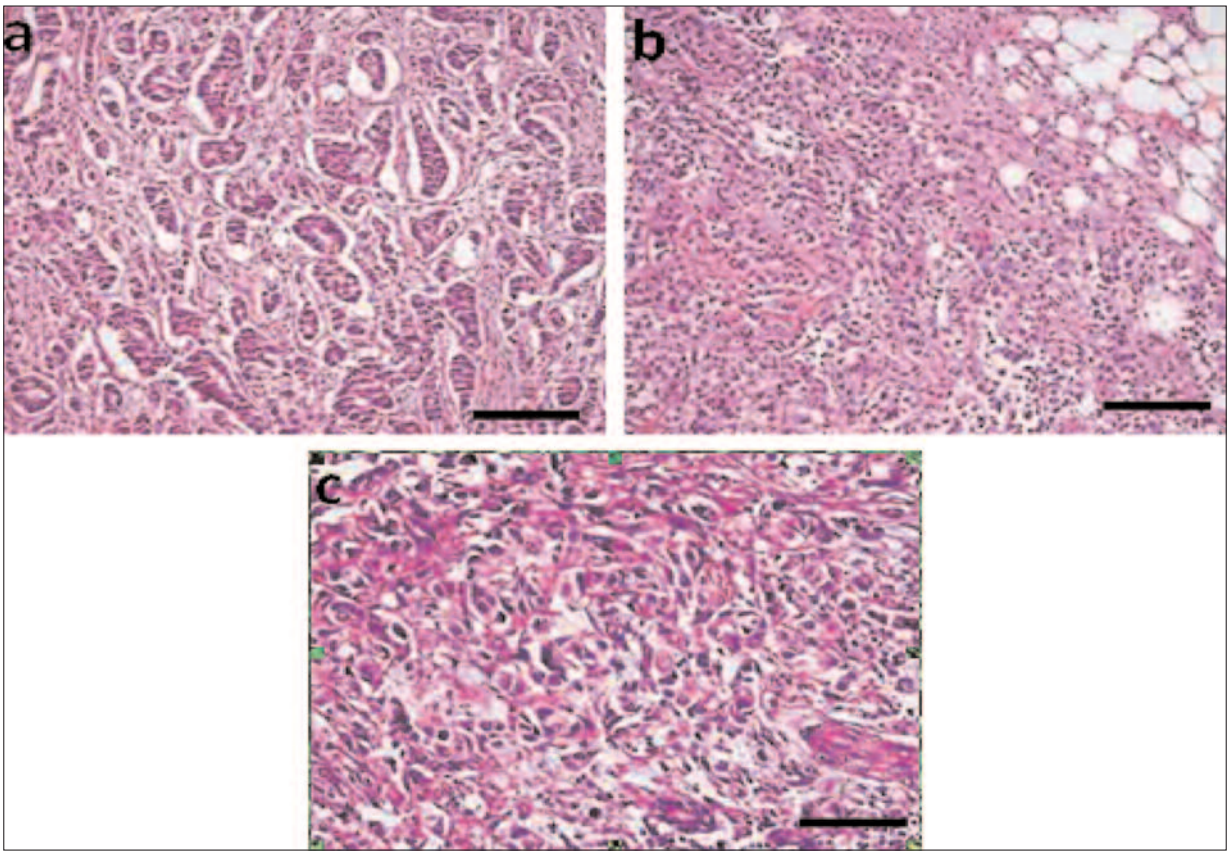


**Figure 1.** Invasive Ductal Carcinoma ***a,*** Grade I, ***b,*** Grade II, ***c,*** Grade III (Hemotoxylin-Eosin (HE), scale bar for ***a, b,*** 200 μm, for ***c,*** 50 μm).

ters of the tumours varied between 0.7 and 14 cm and the mean size was calculated as 3.51 cm. In 14 cases (18.67%), the tumour diameter was smaller than 2 cm (T1), and in 55 cases (73.33%), it was between 2 and 5 cm (T2). In six cases (8%), it was bigger than 5 cm (T3). Furthermore, 46 cases (61.33%) were evaluated as ER-positive and 29 cases (38.67%) as ER-negative (Figure 2a). In addition, 43 cases (57.33%) were evaluated as PR-positive and 32 cases as PR-negative (Figure 2b), while 50 cases (66.67%) were evaluated as cErbB2-positive and 25 as cErbB2-negative (Figure 2c). For Bax, in 59 cases (78.7%), there was no staining, whereas positive-cytoplasmic staining was detected in 16 cases (21.3%; Figure 3a, b). No significant correlation was found between the Bax expression and patient age, tumour diameter or tumour grade. Moreover, there was no statistically significant correlation between Bax and ER, PR and cErbB2 (Table I). Of the 75 cases, 24 (32%) were evaluated as negative and 51 cases (68%) were evaluated as positive for Bcl-2. Staining was localized in the cytoplasm (Figure 3c-e). No significant correlation was found between Bcl-2 and patient age or tumour diameter, and the correlation between Bcl-2 and tumour grade was not statistically significant ($p = 0.08$). There was a significant statistical correlation between ER and Bcl-2 ($p = 0.004$). Moreover, 88.4% of the 43 PR-positive cases (38 cases) were stained positively with Bcl-2. These two parameters were also statistically significant ($p = 0.005$). However, there was no correlation between Bcl-2 and cErbB2 (Table I). With hTERT staining, 41.3% of the 75 cases (31 cases) were negative and 58.7% (44 cases) were positive (Figure 4a, b). The correlation between hTERT and tumour diameter was not statistically significant. Between the tumour grade and the hTERT expression, there was a significant statistical correlation ($p = 0.02$). The correlations between hTERT and ER, as well as between PR and cErbB2, were not statistically significant. Although it was not statistically significant, hTERT positivity was higher in ER-positive cases than in ER-negative cases. Again, although it was not

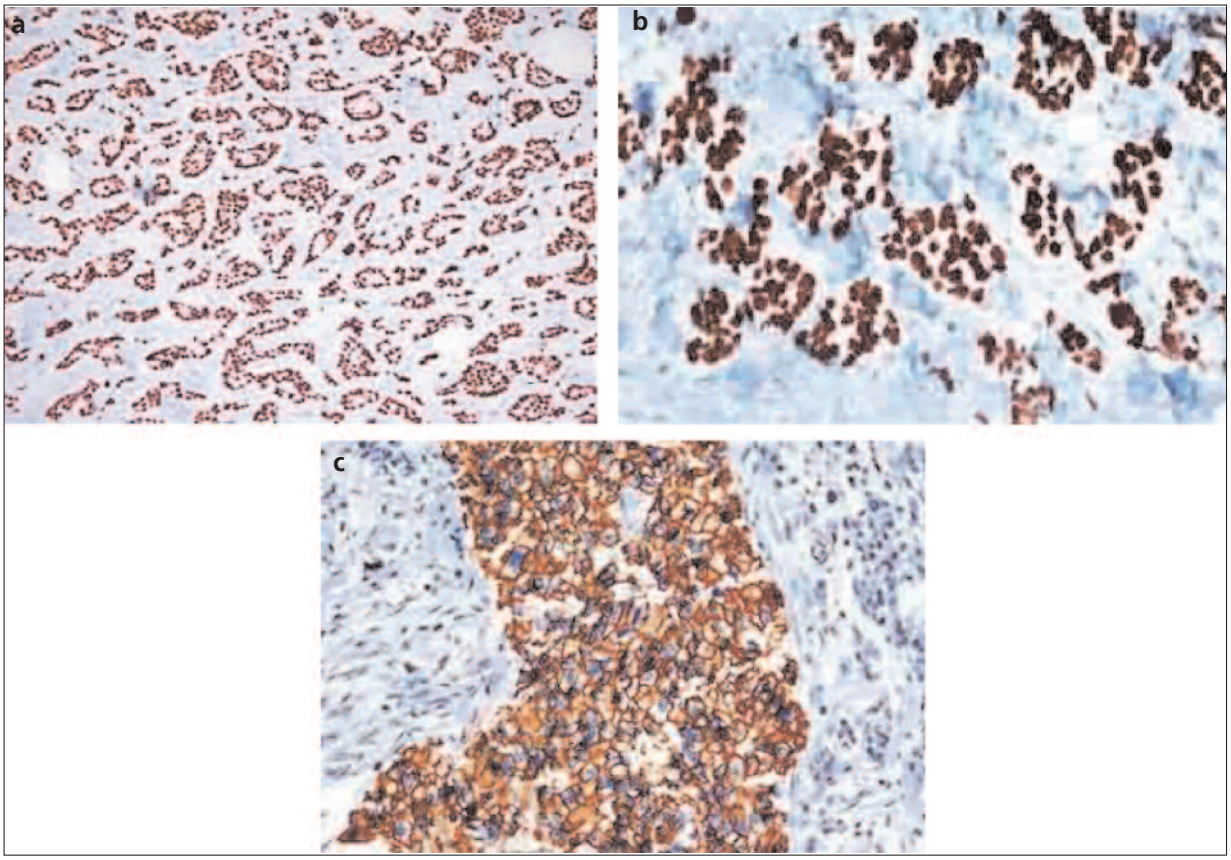


**Figure 2.** Immunostainings. ***a,*** Positivity for estrogen receptor (ER). ***b,*** Positivity for progesterone receptor (PR). ***c,*** Positivity for c-ErbB2 (Immunoperoxidase, hematoxylin counterstain, scale bar for ***a,*** 200 μm, for ***b,*** 100 μm, and for ***c,*** 50 μm).

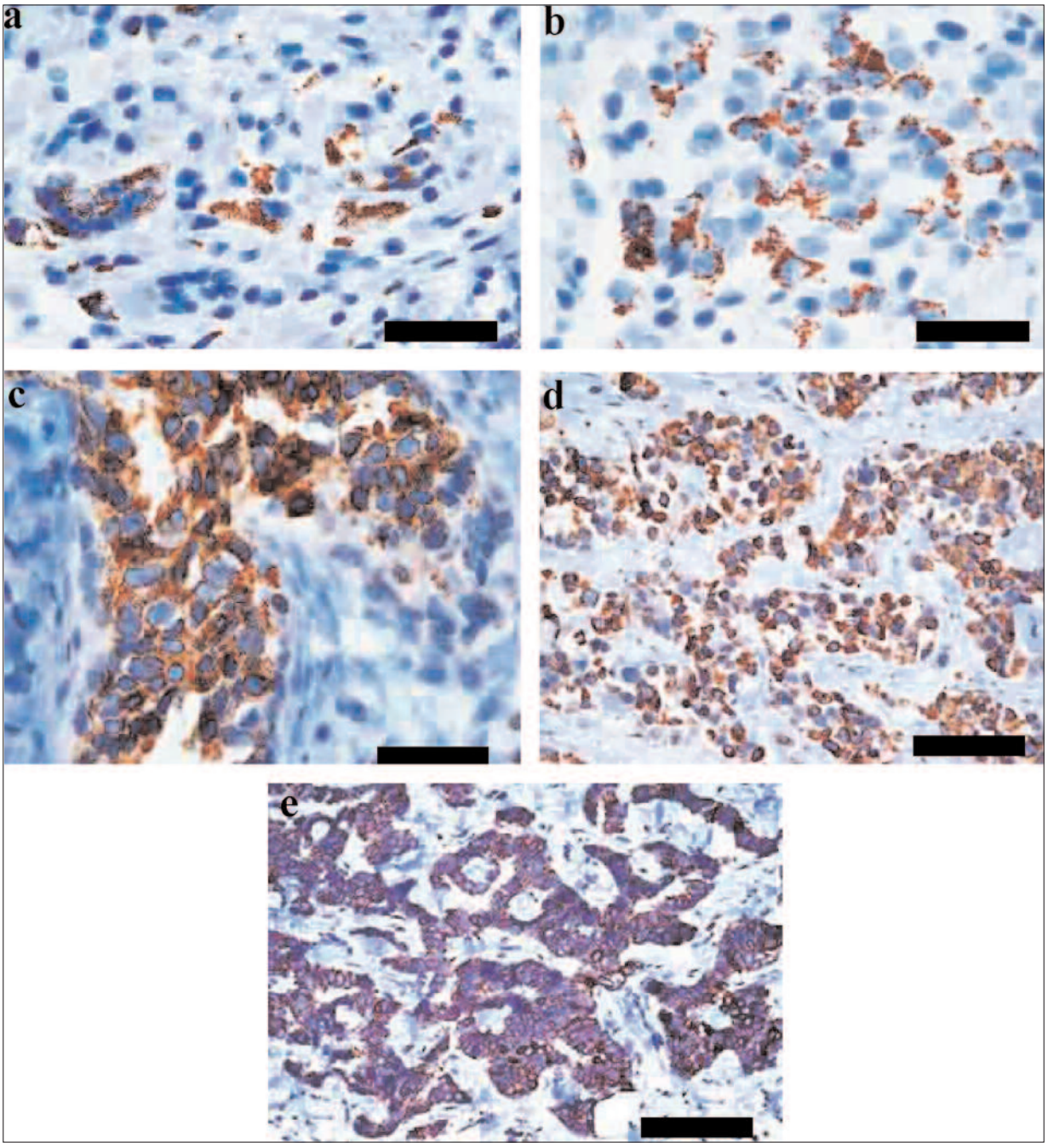


**Figure 3.** Immunostainings. ***a,*** Positivity for Bax, Grade I. ***b,*** Positivity for Bax, Grade II. ***c,*** Positivity for Bcl-2, Grade I. ***d,*** Positivity for Bcl-2, Grade II, e Positivity for Bcl-2, Grade III. (Immunoperoxidase, hematoxylin counterstain, scale bar for ***a, b, c,*** 20 μm and for ***d,*** e 50 μm).

statistically significant, hTERT positivity was higher in PR-positive cases than in PR-negative cases, and hTERT-positivity was higher in cERbB2-positive cases than in cERbB2-negative cases, but it was not statistically significant (Table I). As well, in 53.3% of the 75 cases, no staining was detected, while in 46.7%, positive staining localized in the cytoplasm was seen for BNIP3 (Figure 4c-e). There was quite a significant statistical correlation between BNIP3 and tumour grade ($p = 0.009$). However, there was no correlation between age and tumour diameter. In addition, no correlation was found among ER, PR and cErbB2 (Table I).

**Table I.** Correlations between patient age, tumor diameter, tumor grade, ER status, PR status, cErbB2 status, Bax, Bcl2, hTERT and BNIP3.

| Feature | | % Bax | *p* value | % Bcl2 | *p* value | %hTERT | *p* value | % BNIP3 | *p* value |
|---|---|---|---|---|---|---|---|---|---|
| Age | > 50 | 28.9 | 0.103 | 71.1 | 0.566 | 60.5 | 0.925 | 44.7 | 0.317 |
| | ≤ 50 | 13.5 | | 64.9 | | 59.5 | | 48.6 | |
| Diameters of tumors (cm) | ≤ 2 | 28.6 | 0.528 | 71.4 | 0.954 | 57.1 | 0.924 | 57.1 | 0.464 |
| | 2-01-2005 | 18.2 | | 67.3 | | 60 | | 47.3 | |
| | > 5 | 33.3 | | 66.7 | | 66.7 | | 16.7 | |
| Grade | 1 | 5.9 | 0.197 | 88.2 | 0.084 | 29.4 | 0.02 | 76.5 | 0.009 |
| | 2 | 25 | | 64.6 | | 66.7 | | 41.7 | |
| | 3 | 30 | | 50 | | 70 | | 20 | |
| ER status | Negative | 31 | 0.103 | 48.3 | 0.004 | 72.4 | 0.115 | 96.6 | 0.564 |
| | Positive | 15.2 | 80.4 | | 87 | | 93.5 | | |
| PR status | Negative | 21.9 | 0.921 | 40.6 | 0 | 75 | 0.225 | 93.8 | 0.761 |
| | Positive | 20.9 | | 88.4 | | 86 | | 95.3 | |
| cErbB2 status | Negative | 24 | 0.69 | 72 | 0.6 | 76 | 0.402 | 92 | 0.467 |
| | Positive | 20 | | 66 | | 84 | | 96 | |

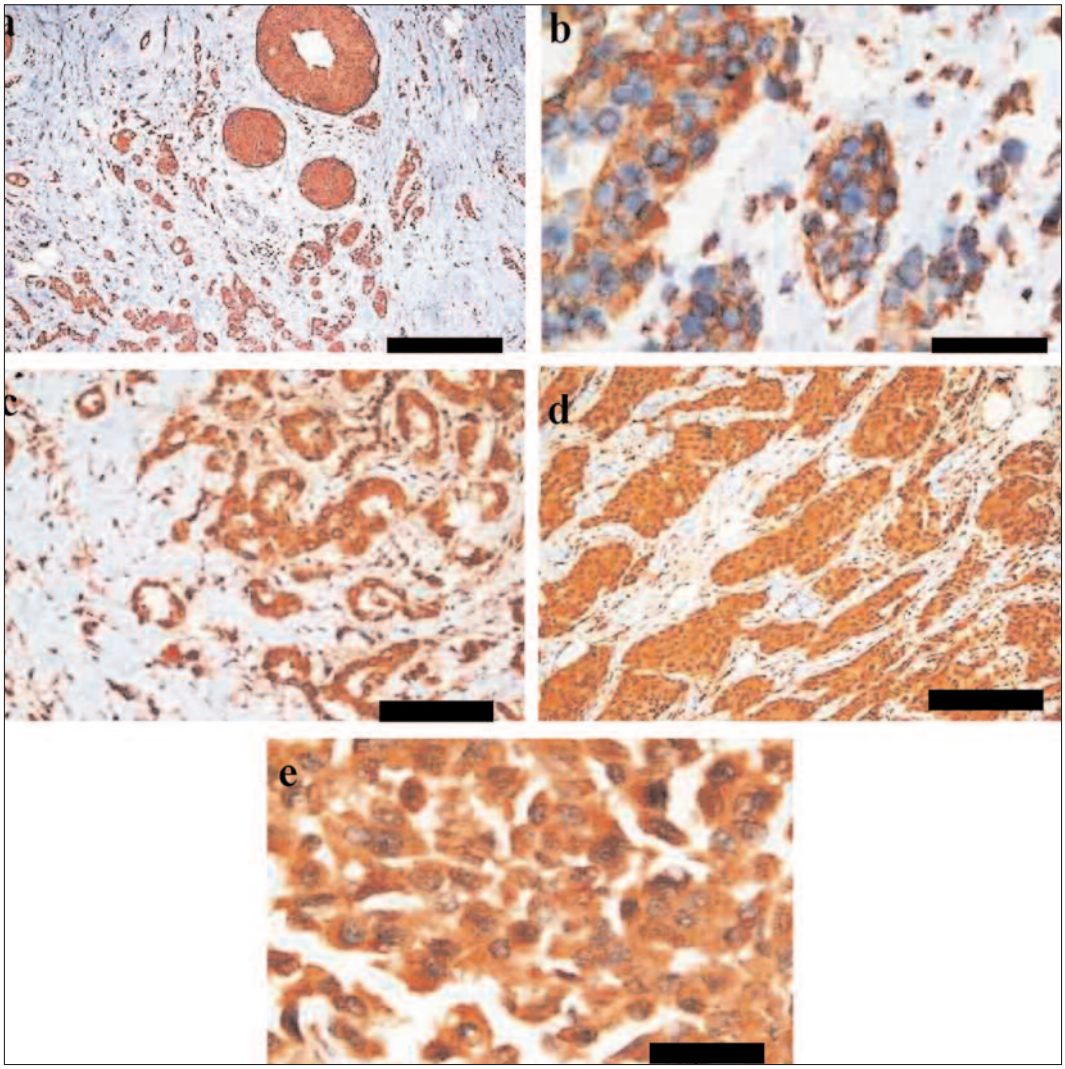


**Figure 4.** Immunostainings. ***a,*** Positivity for hTERT, Grade I and area of ductal carcinoma in situ (DCIS). ***b,*** Positivity for hTERT, Grade II. ***c,*** Positivity for BNIP3, Grade I. ***d,*** Positivity for BNIP3, Grade II, e Positivity for BNIP3, Grade III. (Immunoperoxidase, hematoxylin counterstain, scale bar for ***a, d,*** 200; for ***b,*** e 20 μm and for ***c,*** 100 μm).

Six samples were taken for Western blot. They had a mean age of 52.16 years, and the average tumour diameter was 5.15 cm (2.5 to 7.3 cm). One of the cases was evaluated as grade 3, four as grade 2 and one as grade 1. While Bax in the entire tumour was lower than the control, it showed the least value in only one of our patients with a grade 3 tumour. No significant difference was observed between grades 1 and 2 (Figure 5). Bcl-2 was observed as higher than the control in grade 1 cases, while in all grade 2 and grade 3 cases, it was observed as lower than the control (Figure 6). BNIP3 in grade 3 tumours seemed to be significantly lower than in the control, and it showed the lowest level. No significant difference was observed between grades 2 and 1. In only one case, it was found to be higher than the control (Figure 7). For hTERT, a grade 1 tumour showed a four-fold increase when compared to the control. In grade 3, it was lower than the control. Among grade 2 tumours, only one case was found to be lower than the control (Figure 8). (The control group results are considered as 100 and given a percentage concentration value).

## Discussion

Invasive carcinomas of the breast are the most common cancers in women, following lung cancer, in cancer-related deaths. IDC is the most common histological subtype in this group[11]. The average age at diagnosis in breast tumours is 64 years[17,18]. The average age of the patients includ-

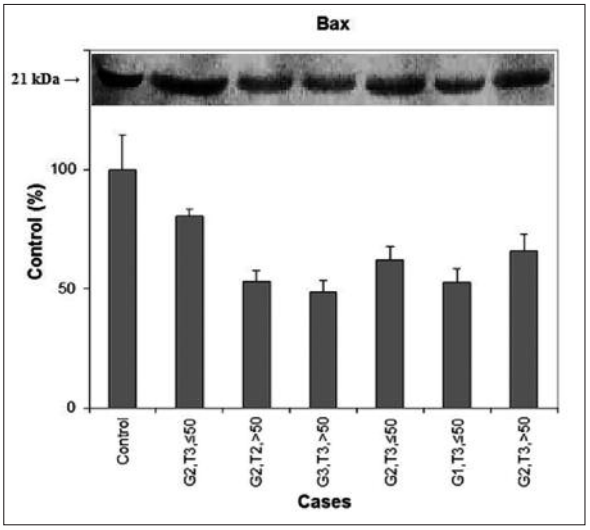


**Figure 5.** Western Blot example for Bax.

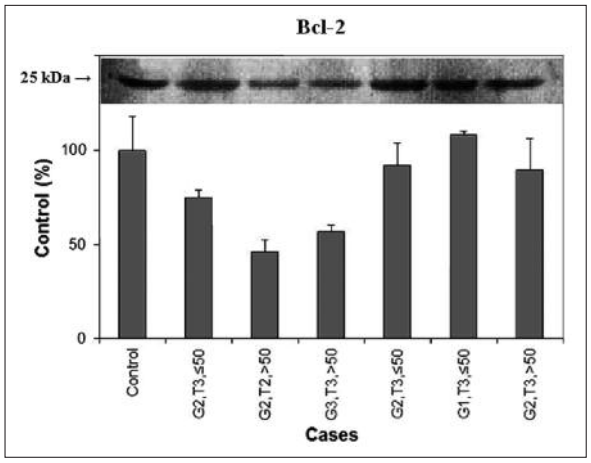


**Figure 6.** Western Blot example for Bcl-2.

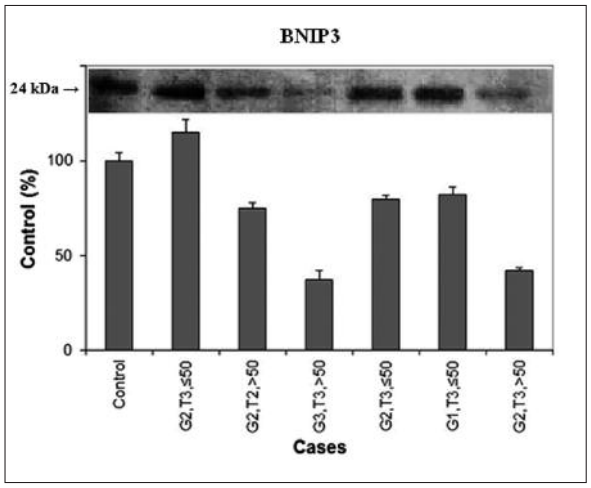


**Figure 7.** Western Blot example for BNIP3.

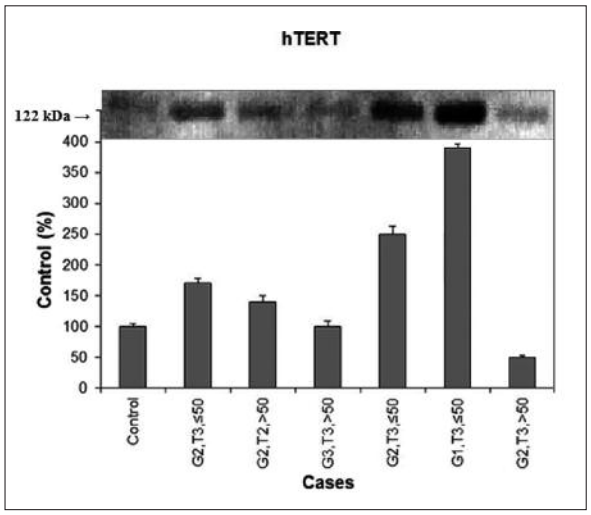


**Figure 8.** Western Blot example for hTERT.

ed in our study is 52.8 years. This number is less than expected, which may well be associated with an increase in the rate of early diagnosis provided by advanced imaging methods. As is known, the diameter of the tumour acts as the central pillar in the staging system and is quite valuable for prognosis. Studies[17,19] reveal that with increasing tumour sizes, average life spans decrease and the prevalence of axillary lymph node metastases increases. In our case, the diameters of the tumours ranged from 0.7 to 14 cm and the average diameter was 3.51 cm. The ER and PR status are very important for determining the treatment response for IDC patients[17]. In our study, there was no statistically significant relationship between ER and PR positivity and age and tumour size, but a significant relationship was observed with grade ($p = 0.050$). ER and PR positivity decreased while the grade increased; therefore, ER and PR positivity may be associated with a better prognosis. The net population of tumour cells depends on the balance between cell proliferation and cell death. The goal of treatment is to reduce cancer cell proliferation and increase apoptosis[20]. The Bcl-2 gene family plays a key role in the regulation of apoptosis. While Bcl-2 suppresses apoptosis, Bax, which is another member of the group, induces apoptosis[2,21]. In various publications[1,15,20,22,23], Bcl-2 positivity in breast cancer was reported to be between 40 and 65%. Similar to these results, the ratio of positive staining with Bcl-2 was determined to be 68% in our study. Bcl-2 positivity is associated with good prognostic factors, such as healthy survival and overall survival period[2,12,22,24]. Kymionis et al[1] reported in their study that Bcl-2 positivity was associated with smaller tumour sizes, while Trere et al[12] reported a weak relationship between Bcl-2 positivity and a small tumour size. In our study, Bcl-2 positivity rates were 71.4%, 67.3% and 66.7% in T1, T2 and T3 tumours, respectively. There was not a statistically significant relationship between Bcl-2 positivity and the tumour size ($p = 0.954$). However, Bcl-2 positivity was reduced as the tumour diameter increased. Many studies determined an association between Bcl-2 and tumour grade[1,12,15,20,22]. In our study, we determined Bcl-2 positivity as 88.2%, 64.6% and 50% in grades 1, 2 and 3 tumours, respectively. These rates were not statistically significant ($p = 0.084$). However, as the degree increased, Bcl-2 positivity was found to be decreased in a manner similar to other studies in the literature. Increased apoptosis in high-grade tumours, as well as the decrease of Bcl-2 positivity as the grade increases, indicate the importance of Bcl-2 in the control of apoptosis. The relationship between ER and PR positivity and Bcl-2 excretion is reported in the literature[1,12,15,20,22,23]. In our study, in 80.4% of the ER-positive and 88.4% of the PR-positive cases, Bcl-2 was positive and this was statistically significant (resp.; $p = 0.004$, $p = 0.00$). The increased release of Bcl-2 in ER- and PR-positive tumours is suggestive of an association between Bcl-2 secretion and high levels of steroid hormones in medium.

Alleged to be associated with poor prognosis, c-ErbB2 was positive in 20 to 30% of breast tumours. In our study, Bcl-2 did not have a significant relationship with c-erbB2 ($p = 0.600$). In the literature, the Bax positivity rate was reported to range between 12 and 75% in breast tumours[15,20,22,23]. Bax positivity in our study is 21.3%. Baccouche et al[23] did not find a significant relationship between age and Bax. In our study, this rate was not sttistically significant ($p = 0.103$). In several studies[1,22], no significant relationship was found between Bax positivity and tumour size and grade. In our study, there was no statistical significance, as well ($p = 0.528$, $p = 0.197$). The study by Kymionis et al[22] shows no relationship between the release of Bax and ER and PR positivity. Similarly, in our study, no significant association was found between Bax and ER, PR and c-erbB2 positivity ($p = 0.103$, $p = 0.921$, $p = 0.690$).

BNIP3 is a member of the Bcl-2 family and is increased under hypoxic conditions[4,5]. The immunohistochemical studies show that, in DCIS and invasive breast carcinoma, the BNIP3 levels were found to be significantly higher in the cytoplasms and the nuclei of the neoplastic cells when compared to normal breast tissue. In our study, no significant relationship was shown between patient age and tumour size with the release of BNIP3 ($p = 0.317$, $p = 0.464$). BNIP positivity was found to be 76.5%, 41.7% and 20% in grades 1, 2 and 3 tumours, respectively, and there was a statistically significant relationship between tumour grade and BNIP3 release ($p = 0.009$). The significant relationship between BNIP3 and grade, which has an importance in determining the prognosis of IDC, shows that BNIP3 is a valuable marker and can provide valuable information about the prognosis.

In our study, no significant correlation was found between BNIP3 and ER, PR and c-erbB2 positivity ($p = 0.564$, $p = 0.761$, $p = 0.467$). In

addition, hTERT, which is decisive for the activity of telomerase, is not present in normal somatic cells, but its release is increased in DCIS, such as pre-invasive lesions of the breast and in breast cancer[9,25]. Several studies show hTERT positivity in IDC to be between 50 and 90%[26,27]. In our study, hTERT positivity was found to be 58.7%. Few studies reported no significant relationship between patient age and hTERT[10]. In our study, no significant correlation was found between hTERT and age and tumour size ($p = 0.925$, $p = 0.924$). Mokbel et al[28] reported that telomerase-positive tumours were mostly grade 3, so there was a significant relationship between grade and hTERT positivity[29,30]. In our study, hTERT positivity significantly increased parallel to the increase in grade ($p = 0.020$). The release of hTERT is increased in pre-invasive breast lesions, such as DCIS, so telomerase activity is supposed to be effective in the early period of carcinogenesis[31]. Telomerase activity is controlled by hormone-dependent mechanisms. Some studies[25,32,33] state a significant relationship between telomerase activity and ER and PR positivity, while some state none[10,30]. We also found no significant relationship in our study ($p = 0.115$, $p = 0.225$). Divella et al[33] reported a significant relationship between high levels of hTERT and high levels of HER2/Neu, while Kalogeraki et al[29] suggested a significant relationship between telomerase activity and HER2 release. In our study, hTERT positivity was found to be 84% in c-erbB2-positive cases, while it was 76% in c-erbB2-negative cases. Although hTERT positivity in c-erbB2-positive cases seems to be higher than in c-erbB2-negative cases, it is not statistically significant ($p = 0.402$).

According to the immunohistochemistry method in a grade 1 tumour, Bcl-2 positivity was as high as 88.2%, and while the grade increased, Bcl-2 positivity decreased. In our western blot study, the only grade 1 case showed a higher Bcl-2 level than the control, while in all the other grade 2 and 3 cases, Bcl-2 levels were lower than in the control. Therefore, the important role of Bcl-2 in apoptosis, as obtained by IHK method, has also been confirmed by the Western blot method.

According to the immunohistochemical findings, there was no significant relationship between Bax positivity and the grade of tumour, but there was a slight increase in Bax positivity when the grade increased. In the western blot method, Bax was low in all cases with respect to the control, except in one grade 3 case, which showed the lowest value. Somewhat similar results were obtained in other tumours. Different results for Bax with IHK and the western method suggest that its pro-apoptotic effect is not done alone but possibly with the help of many genes or proteins.

The significant decrease of BNIP3 revealed by the western blot method in a grade 3 tumour – which is actually the lowest level – with respect to control, is consistent with our statistically significant results obtained by the IHK method. Thus, the western blot method supports our findings, telling us that BNIP3 decreases significantly as the grade increases, and it is effective in early tumoural staging; hence, it may be a valuable marker for prognosis.

In the western blot method, hTERT increased in grade 1 tumours by about four times compared to the control. This increase is also compatible with our results obtained through IHK, leading to the suggestion that hTERT can be used as a cancer marker in the IDC. According to our IHK results, the hTERT level increases with the increase in tumour grade, but these results were not supported by Western blot.

Consequently, IDC telomerase activity is believed to be related to tumour growth. The hTERT positivity of the DCIS areas suggests that telomerase activity is efficacious in the early stages of tumour development. As hTERT is negative in normal breast tissue, it is suggested that it can be used as an important tumour marker. The effect of hTERT in the early stages of tumours has also been confirmed by the Western blot method.

While the increased telomerase activity is associated with tumour grade, the reduction in BNIP3 is associated with apoptosis. The positivity of both of them in the DCIS areas suggests that apoptosis is also effective in the early stages of tumour development along with telomerase activity.

As the tumour stage increased, a decrease in Bcl-2 and BNIP3 and an increase in Bax were determined. While the values obtained for Bcl-2 and Bax were not statistically significant, the values of BNIP3 were significant. This finding showed that BNIP3 is more valuable in determining the prognosis or treatment of IDC as compared to Bcl-2. In addition, the western blot method also confirmed BNIP3 as a valuable marker for determining a prognosis. However, though not statistically significant, the increase of apoptosis in high-grade tumours and the decrease in Bcl-2 positivity as the grade increases

support the importance of Bcl-2 in the control of apoptosis, which is also supported by the results obtained by the western blot method.

As the stage increases, ER and PR positivity significantly decrease as well as Bcl-2, indicating that the hormonal status in IDC plays an important role in controlling apoptosis. Moreover, in ER- and PR-positive tumours, the Bcl-2 release is significantly higher, which is suggestive of the association between the Bcl-2 expression and a high level of steroid hormones.

## Conclusions

If some treatment options can be developed to suppress the increase in telomerase activity and hypoxic cell death, tumour progression may be prevented. If these changes can be detected at the molecular level in an individual, promising results may be achieved with early-stage carcinomas.

### Conflict of Interest

The Authors declare that there are no conflicts of interest.

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