CORRELATION BETWEEN EXPRESSION OF BCL-2, BAX & P53 AND HISTOLOGICAL ALTERATIONS IN BREAST CANCER OF EGYPTIAN PATIENTS

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ABSTRACT

In Egypt, breast cancer represents approximately 33% of all female cancers and 29% of cancer cases treated at the National Cancer Institute. The present work aimed to determine the differences between normal breast tissue, fibroadenoma and invasive ductal carcinoma tissues using histopathological, histochemical (PAS reaction was used for demonstration of polysaccharides and mercury bromophenol blue method for demonstration of total proteins), immunohistochemical studies (using antibodies directed against ER, PR, p53, Bax and Bcl-2) as well as DNA fragmentation assay. Sixty female breast specimens were obtained from Pathology Department, National Cancer Institute, Cairo University from 2012 to 2014. A noticeable difference in the content of polysaccharides and proteins in the three studied groups (normal, fibroadenoma and invasive ductal carcinoma) was detected. Tumor specimens showed lowest polysaccharides and highest proteins content. Out of 40 invasive ductal carcinoma (IDC) cases, Bcl-2 expressed in 24 cases (60%) and Bax expressed in 16 cases (40%). Also, 30% and 40% of the IDC specimens showed positive ER and PR expression; respectively. Total optical density in IDC specimens was statistically increased due to fragmentation of DNA. It was concluded that normal breast tissues contained the highest polysaccharides contents, while fibroadenoma specimens showed moderate content and the lowest content was in IDC specimens. The total proteins in the invasive ductal carcinoma specimens showed significant increase compared with fibroadenoma and normal specimens. There was significant increase in Bcl-2, Bax and p53 expression in invasive ductal carcinoma. There was a marked decrease in ER expression in both fibroadenoma and invasive ductal carcinoma than normal breast. There was high DNA fragmentation in invasive ductal carcinoma.

KEYWORDS: Breast cancer, Histology, Histochemistry, Immunohistochemistry, Bcl-2, Bax, p53, ER, PR and DNA fragmentation.

INTRODUCTION

Breast cancer is the second most common diagnosed cancer; accounting 23% of the total diagnosed cancer cases and ranks as the fifth cause of death (14%) (Ferlay *et al*., 2010; Jemal *et al*., 2011). According to the results of the National Cancer Registry Program of Egypt 2008 – 2011, Ibrahim *et al*. (2014) estimated that the most frequent cancers site in female is the breast (32.04%). Allam and AbdElaziz (2012) reported that 29% of the cancer cases treated at the national cancer institute was breast cancer. Breast cancers are histopathologically divided into epithelial lesions (benign and malignant) which is further divided into carcinoma in situ and invasive carcinoma, epithelial/stromal (biphasic), metastatic and stromal tumors (Al-Nafussi *et al*., 2005).

Analysis of different genes isolated from different breast cancers divided it into luminal (estrogen or progesterone receptor) positive; human epidermal growth factor receptor 2 (HER2)-like (mainly ER- and HER2+); basal-like (mainly estrogen receptor negative, progesterone receptor negative, and HER2 negative) also called tripple

negative and triple positive, positive for estrogen receptors, progesterone receptors and HER2 (Perou *et al*., 2000).

B-cell lymphoma 2 (Bcl-2) family includes many proteins, which can promote either cell survival, such as Bcl-2 or cell death, like Bcl-2-associated X protein (Bax) (Adams and Cory, 1998; Reed, 1998). The balance between expressions of these genes regulates the cell cycle and apoptosis. Also, tumor suppressor protein (p53) regulates cell cycle and apoptosis. Presently, p53 is known to play a key role in practically all types of human cancers, and the mutation or loss of the p53 gene can be identified in more than 50% of all human cancer cases worldwide. When DNA damaged, the level of p53 protein rise, p53 causes the cell to delay its entry into S phase until the damage is repaired. When the damage is too severe to repair p53 is involved in stimulating an apoptotic response.

The present work was performed to correlate between expression of Bcl-2, Bax and p53 and histological

alterations in different types of breast cancer of Egyptian patients.

PATIENTS AND METHODS
Patient groups
Sixty female breast specimens were obtained from Pathology Department, National Cancer Institute, Cairo University from 2012 to 2014. The study met the criteria of the Ethics committee of the institution. The patients were divided into three groups;
Group 1: Ten specimens were used as control; they were taken from tissues adjacent to fibroadenoma.
Group 2: Ten specimens of fibroadenoma.
Group 3: Forty specimens with invasive ductal carcinoma.

Clinical data
Clinical data were obtained from the patient's medical records including patients age, tumor size and grade of the tumor.

Histological studies
Tissues were fixed in 10% neutral formalin, dehydrated through ascending series of alcohol, then cleared in two changes of xylene and embedded in paraffin. Sections of 5 μm thickness were cut using rotary microtome for histological, histochemical and immunohistochemical studies.

Histochemical studies
Periodic acid Schiff (PAS) reaction was used for demonstration of polysaccharides (Kiernan, 1981); the positive tissues stained magenta, and mercury bromophenol blue method for demonstration of total proteins (Troyer, 1980); the positive tissues stained deep blue.

Immunohistochemical studies
Antibodies directed against ER, PR (Taylor and Kledzik, 2002) as well as p53, Bax and Bcl-2 (Hsu et al., 1981)

were used. Five micron thickness sections were deparaffinized and rehydrated followed by blocking endogenous peroxidase activity in 1.0% hydrogenperoxide in PBS for 15 min. and antigen retrieval was performed by microwave heating in citrate buffer, pH 6. Streptavidin-biotin immunoperoxidase method was used for each section (Dako, Universal LSAB‑2 kit). Sections were incubated with monoclonal primary mouse antibodies (Neo Markers, 1:200) for 30 minutes at room temperature. 3,3diaminobenzidine tetrahydrochloride (DAB) was used as a chromogen and sections were counterstained with Mayer's hematoxylin before mounting. Positive cells were counted in 5 high power field.

DNA Fragmentation Assay
As a measure of apoptotic DNA fragmentation, DNA was extracted according to Aljanabi and Martines (1997) and modification was introduced by Hassab El-Nabi and El hassaneen (2008). The photographed gel is analyzed by Gel Pro-program.

Statistical analysis
Data were analyzed statistically for normal distribution (student' t test) and homogeneity of variances (Levene test) using statistical program of social sciences (SPSS) software for windows, version 11. Differences were considered significant whenever $p<0.05$.

RESULTS
Clinicopathological data
Data presented in table 1 showed that the mean age of female patients of control, fibroadenoma and IDC groups was 38.6 ± 3.93, 26.5 ± 8.38 and 46.17 ± 1.15 years, respectively. In IDC group, the number of patients with tumor size < 2cm was 22 (55%) and number of patients with tumor size ≥2 cm was18 (45%). Number of patients with grade 1, grade 2, and grade 3 were zero, 37, and 3 respectively.

Table 1: Patient's characteristics in the different patient groups.

Patient groups	Number of patients	Age/years (Mean ± SE)	Tumor size in cm	Number of patients in each grade		
				Grade 1	Grade 2	Grade 3
Control	10	38.6 ± 3.93	0	0	0	0
Fibroadenoma	10	26.5 ± 8.38	0	0	0	0
IDC	40	46.17 ± 1.15	<2=22 ≥2=18	0	37	3

Histopathological observations
Examination of normal breast sections stained with hematoxylin and eosin showed the lobule, the acini, the ducts and the stroma .The lobule was surrounded from outside and inside by connective tissue (stroma); interlobular and intralobular connective tissue, respectively which was denser in outside than inside. The intralobular connective tissue was rich with many types of cells while the interlobular connective tissue was thicker, denser and relatively noncellular (collagen fiber and adipose tissue) (Fig.1A). Each acinus is enveloped

by basement membrane and lined by two distinct types of cells arranged in spiral fashion; cuboidal or low columnar epithelial and myoepithelial cells (Fig.1B).

Microscopic examination of fibroadenoma showed intracanalicular fibroadenoma, in which the epithelial elements form slit-like ductal structures. The epithelial cells lining the intracanalicular fibroadenoma were irregular in shape and the stroma was densely fibroblastic (Fig. 1C).

Examination of invasive ductal carcinoma showed that it consisted of irregularly shaped tightly or loosely cohesive groups of pleomorphic tumor cells. The majority of these cells had moderate amounts of eosinophilic cytoplasm and pleomorphic nuclei with a coarse chromatin pattern (Fig.1D).
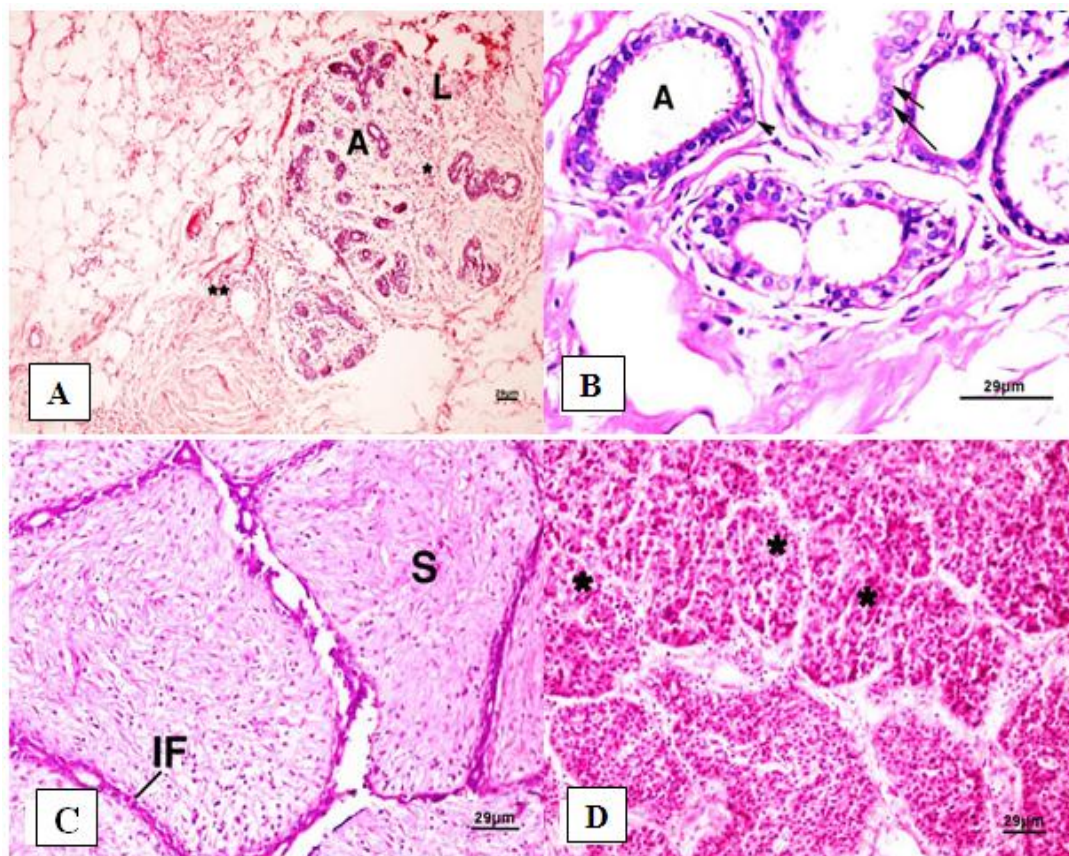

Fig. 1: Photomicrographs obtained from breast tissues stained with H & E: A & B) normal breast tissue showing the lobule (L), acini (A), intralobular connective tissue (*) and interlobular connective tissue (**), basement membrane (long arrow), cuboidal cell (short arrow) and myoepithelial cell (arrowhead). C) intracanalicular fibroadenoma (IF) with densely fibroblastic stroma (S). D) IDC tissue showing tightly or loosely irregular shaped cohesive groups (*) and fibrous tissue (F).

Histochemical observations
Normal breast tissues contained the highest polysaccharide contents, while fibroadenoma specimens showed moderate content and the lowest content was observed in IDC specimens (Figs. 2A-C).

Concerning the total protein contents, the invasive ductal carcinoma specimens showed significant increase compared with fibroadenoma and normal specimens (Figs. 3A-C).
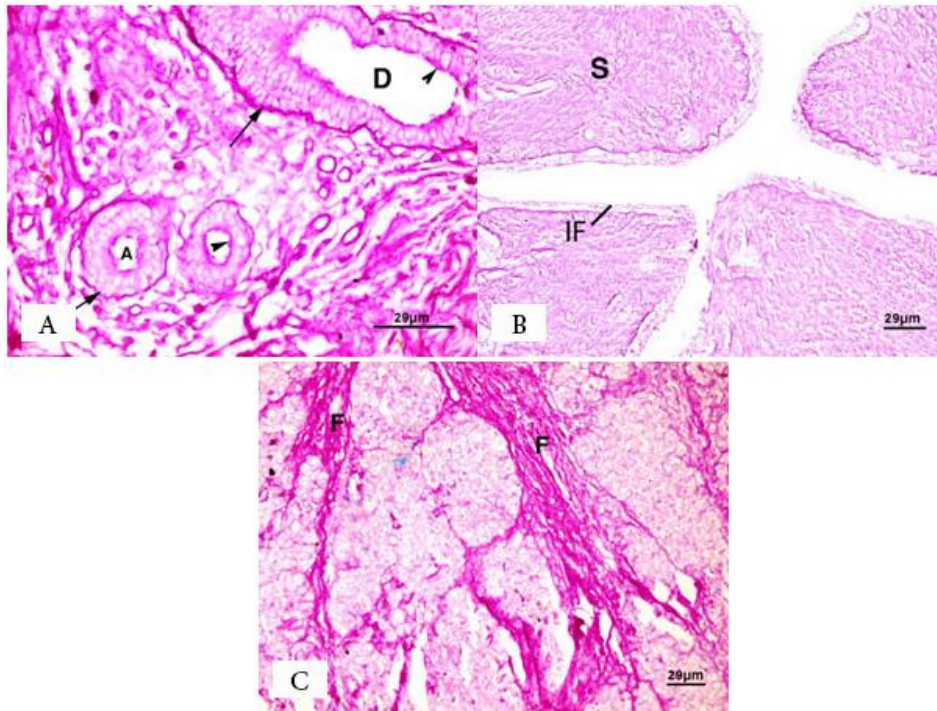
Fig. 2: Photomicrographs obtained from breast tissues (PAS reaction): A) normal breast tissue showing deep purple color in the basement membrane (arrows) of the acini (A), ducts (D), brush borders (arrowheads) and interlobular connective tissue, week reaction in the cytoplasm of the epithelial cells and negative reaction in the nuclei of these cells, **B)** intracanalicular fibroadenoma (IF) showing deep purple color in the membrane surrounding the ductal structure, moderate in the stroma (S), faint in the cytoplasm of the epithelial cells and negative reaction in the nuclei, **C)** IDC tissue showing deep purple color in the fibrous tissue (F), faint color in the cytoplasm of the tumor cells and negatively stained nuclei.
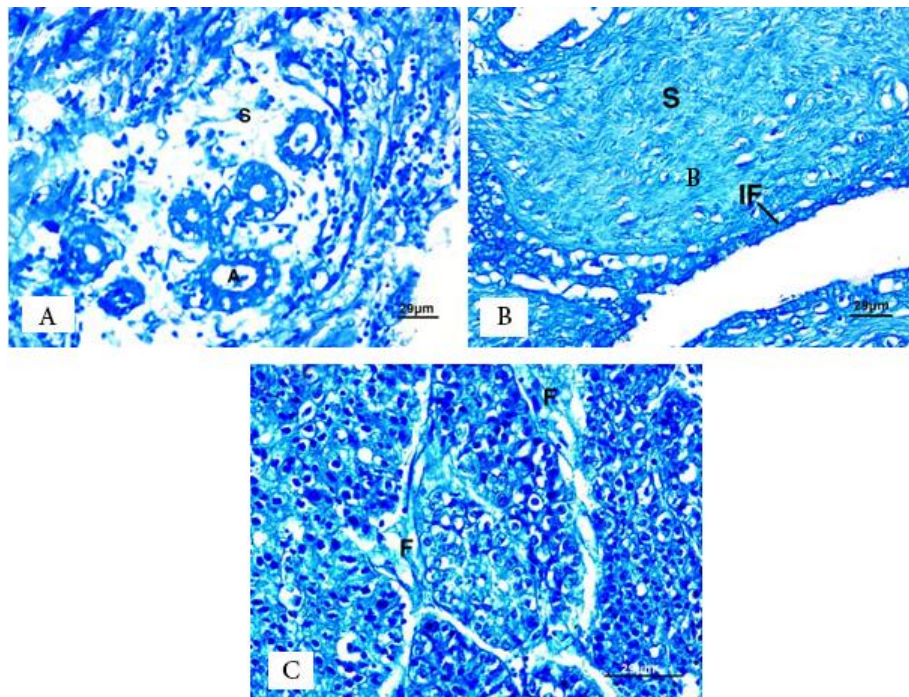

Fig. 3: Photomicrographs obtained from breast tissues (Bromophenol blue): A) normal breast tissue showing dense blue color in the cytoplasm and nuclei of the epithelial cells lining the acini (A), moderate blue color in the stroma (S) and densely stained stromal cells, **B)** intracanalicular fibroadenoma (IF) showing high protein content in the cytoplasm and nuclei of the epithelial cells lining the ductal structures and moderate blue color in the stroma (S), **C)** IDC tissue showing deeply stained nuclei and moderate blue color in the cytoplasm of the majority of the tumor cells and a faint color in the fibrous tissue (F).

Immunohistochemical observations

The number and percentage of cases that showed positive or negative expression for Bcl-2, Bax, p53, ER and PR in the different patient groups were expressed in table 2.

1. Expression of Bcl-2, Bax, p53

Examination of immunostaining preparation of control, fibroadenoma tissue showed negative expression Bcl-2, Bax and p53 (Figs. 4, 5A-C, 6A&B and 7A&B). There was highly significant increase in Bcl-2, Bax and p53 expression in invasive ductal carcinoma in comparison with the other groups (Figs.4, 5D, 6D and 7D).

Table 2: Expression of Bcl-2, Bax, p53, ER and PR in the different patient groups (Number and percentage).

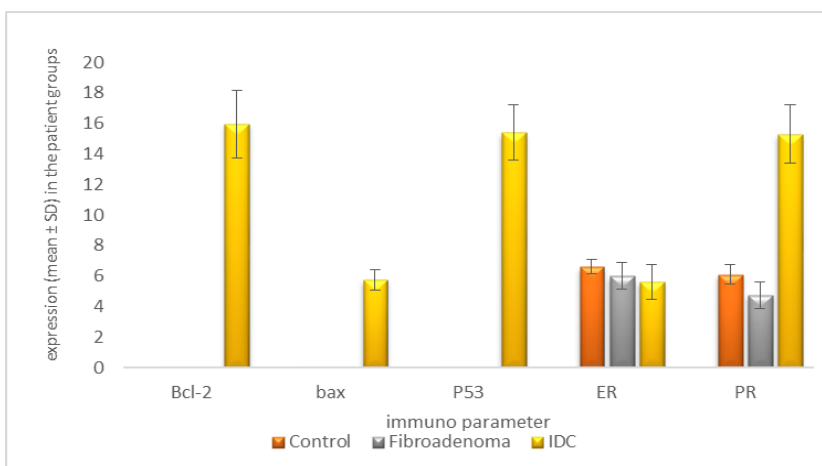| Immune parameter / Patient group		Control	Fibroadenoma	IDC
Bcl-2	+ve	0	0	24(60%)
	-ve	10(100%)	10(100%)	16(40%)
Bax	+ve	0	0	16(40%)
	-ve	10(100%)	10(100%)	24(60%)
P53	+ve	0	0	18(45%)
	-ve	10(100%)	10(100%)	22(55%)
ER	+ve	10(100%)	10(100%)	12(30%)
	-ve	0	0	28(70%)
PR	+ve	10(100%)	10(100%)	16(40%)
	-ve	0	0	24(60%)

Fig. 4: Expression of Bcl-2, Bax, p53, ER and PR in the different patient groups.

Immunohistochemical correlation between Bcl-2 and Bax or p53

The number of cases that showed positive Bcl-2 expression and also were p53 positive was 15 (37.5%) out of 40 IDC cases; the relationship was direct statistically significant ($p<0.0001$). In addition, the number of cases that showed positive Bcl-2 and were Bax positive were 9 (22.5%) out of 40 IDC cases. The relationship was indirect statistically significant ($p<0.0001$) (Table 3).

Table 3: Immunohistochemical correlation between Bcl-2 and Bax or p53.

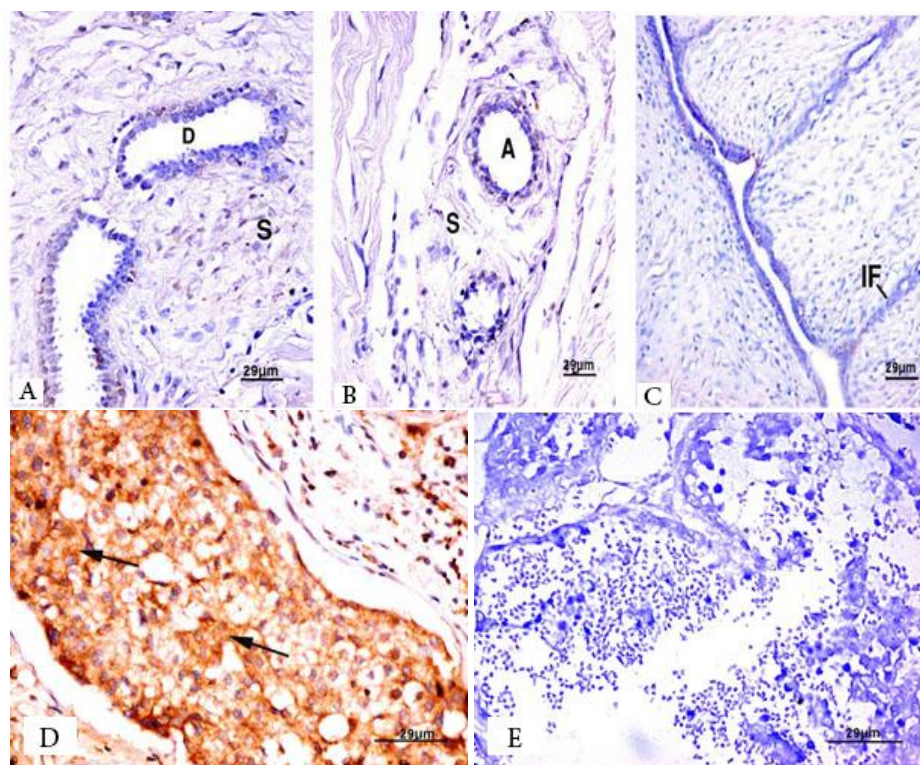| Parameters		Bcl-2				Total	*P* value
		+ve		-ve			
		No	%	No	%		
p53	**+ve**	15	37.5	3	7.5	18	($p<0.0001$)
	-ve	9	22.5	13	32.5	22	
Bax	**+ve**	9	22.5	7	17.5	16	($p<0.0001$)
	-ve	15	37.5	9	22.5	24	

Fig. 5: Photomicrographs obtained from breast tissues (Bcl-2 immunostain, counterstained with hematoxylin): A & B) normal breast tissue showing negative Bcl-2 expression in the cytoplasm of the epithelial cells lining the ducts (D) the acini (A) and the stroma (S), C) intracanalicular fibroadenoma (IF) tissue showing negative Bcl-2 expression, D) IDC tissue showing positive Bcl-2 expression in the cytoplasm (arrows) of the tumor cells, E) IDC tissue showing negative Bcl-2 expression in the cytoplasm of the tumor cells.
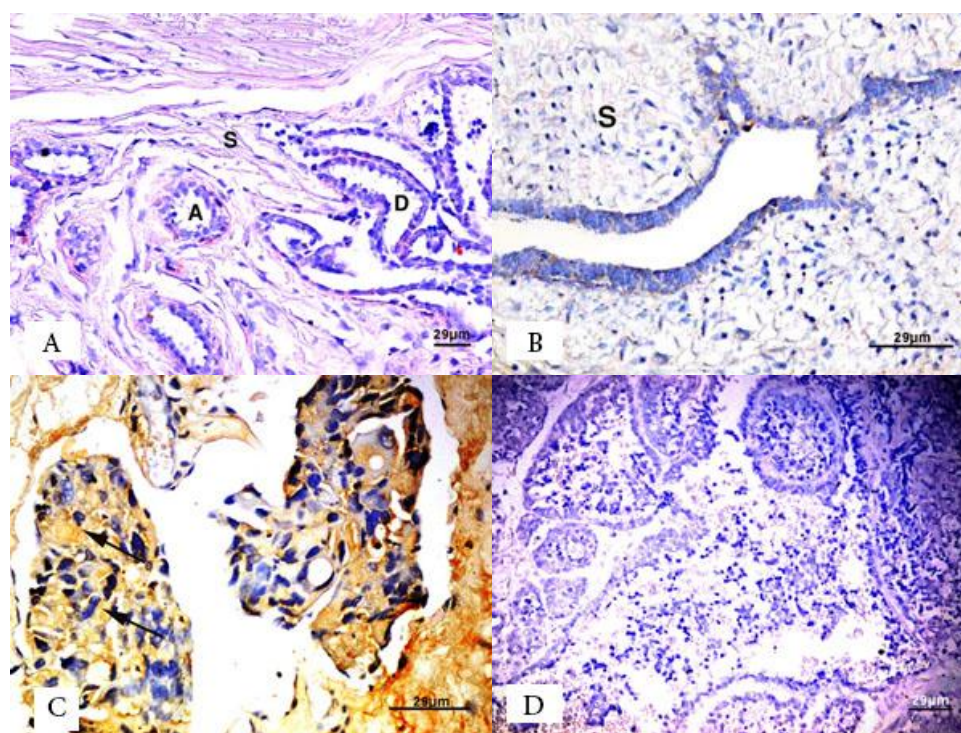

Fig. 6: Photomicrographs obtained from breast tissues (Bax immunostain, counterstained with hematoxylin): A) normal breast tissue showing negative Bax expression in the cytoplasm of the epithelial cells lining both the acini (A) and the ducts (D) and the stroma (S), B) intracanalicular fibroadenoma (IF) tissue showing negative Bax expression in the cytoplasm of the epithelial cells and in the stroma (S), C) IDC tissue showing positive Bax expression in the cytoplasm (arrows) of the tumor cells, D) IDC tissue showing negative Bax expression in the cytoplasm of the tumor cells.
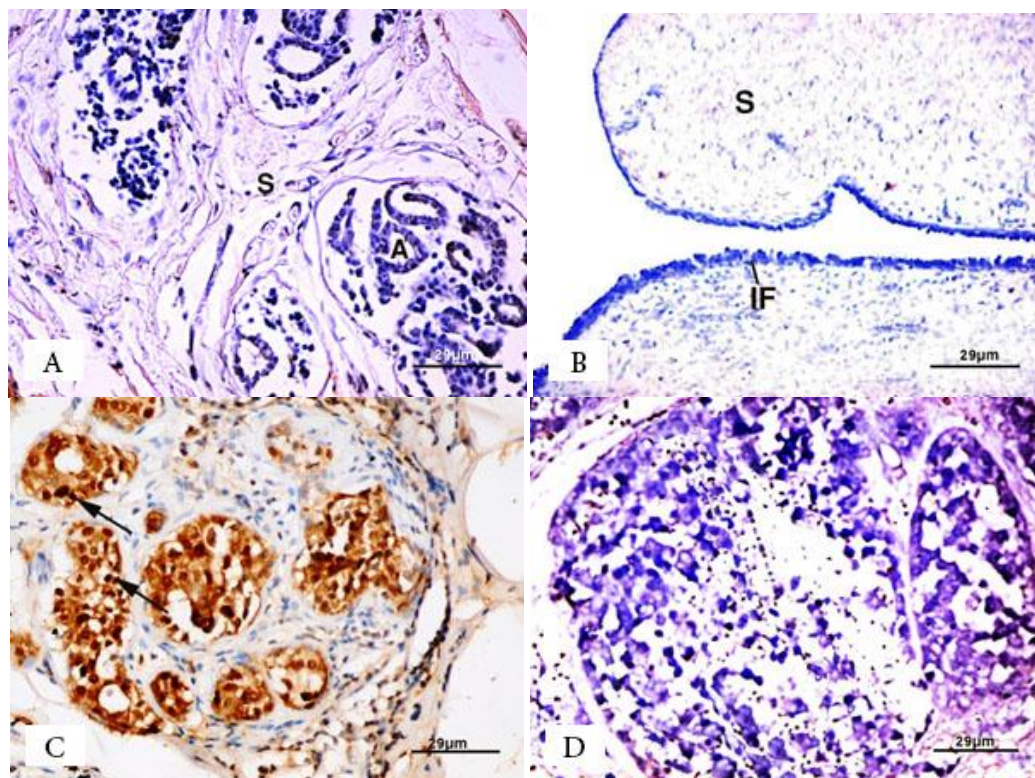
Fig. 7: Photomicrographs obtained from breast tissues (p53 immunostain, counterstained with hematoxylin): A) normal breast tissue showing negative p53 expression in the nuclei of the epithelial cells line the acini (A) and the stroma (S), B) intracanalicular fibroadenoma (IF) tissue showing negative p53 expression in the nuclei of the epithelial cells and in the stroma (S), C) IDC tissue showing positive p53 expression in the nuclei of the tumor cells (arrows), D) IDC tissue showing negative p53 expression.

2. Expresssion of ER and PR

There was a marked decrease in ER expression in both fibroadenoma and invasive ductal carcinoma than normal breast tissue. The reduction of ER expression was highly significant in invasive ductal carcinoma (Figs.4 and 8A-C). In contrast, PR expression showed significant increase in invasive ductal carcinoma group compared with normal breast group, while PR expression showed slight decrease in fibroadenoma group than normal breast group (Figs.4 and 9A-C).

Immunohistochemical correlation between p53 and estrogen or progestron receptors

Out of 40 cases, 4 cases (10%) showed positive p53 expression and were also ER positive. The cases that showed positive p53and PR positive were 7(17.5%). The relationship was indirect statistically significant ($p<0.0001$) (Table 4).

Table 4: Immunohistochemical correlation between p53 and estrogen or progestron receptors.

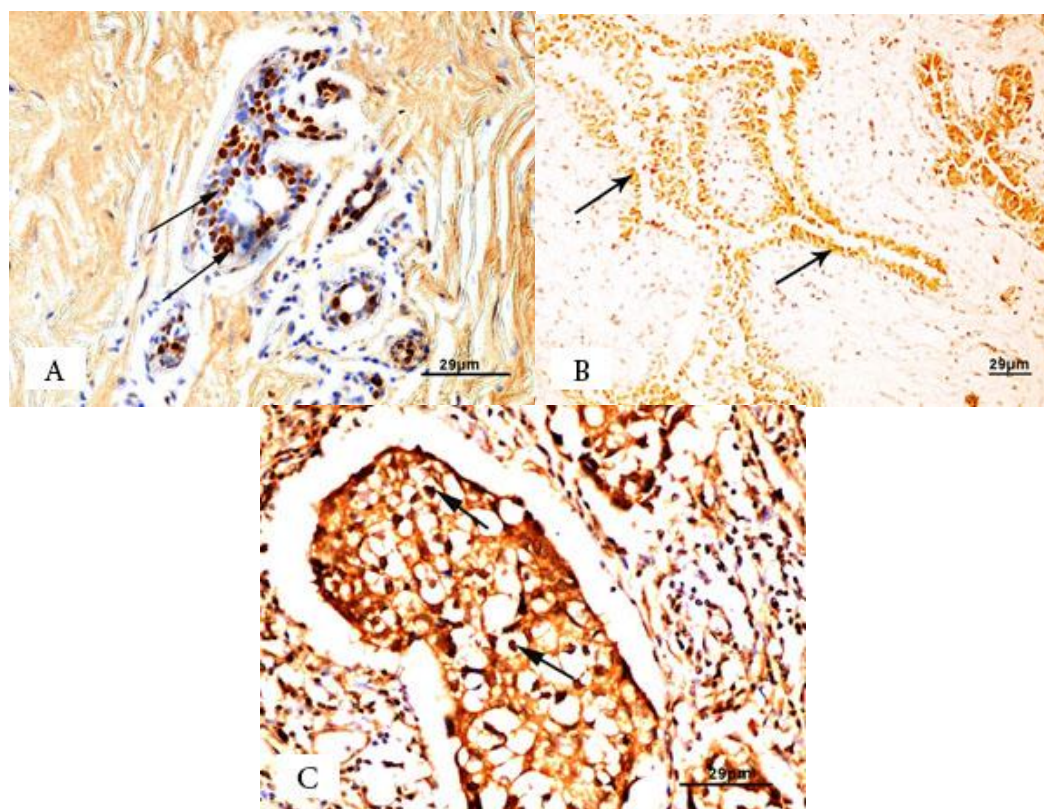| Parameters		p53				Total	*p* value
		+ve		-ve			
		No	%	No	%		
ER	+ve	4	10	8	20	12	($p<0.0001$)
	-ve	14	35	14	35	28	
PR	+ve	7	17.5	9	22.5	16	($p<0.0001$)
	-ve	11	27.5	13	32.5	24	

Fig. 8: Photomicrographs obtained from breast tissues (ER immunostain, counterstained with hematoxylin): A) normal breast tissue showing positive ER expression in the nuclei of the epithelial cells (arrows), B) intracanalicular fibroadenoma tissue showing positive ER expression in the nuclei of the epithelial cells (arrows), C) IDC tissue showing positive ER expression in the nuclei of the tumor cells (arrows).
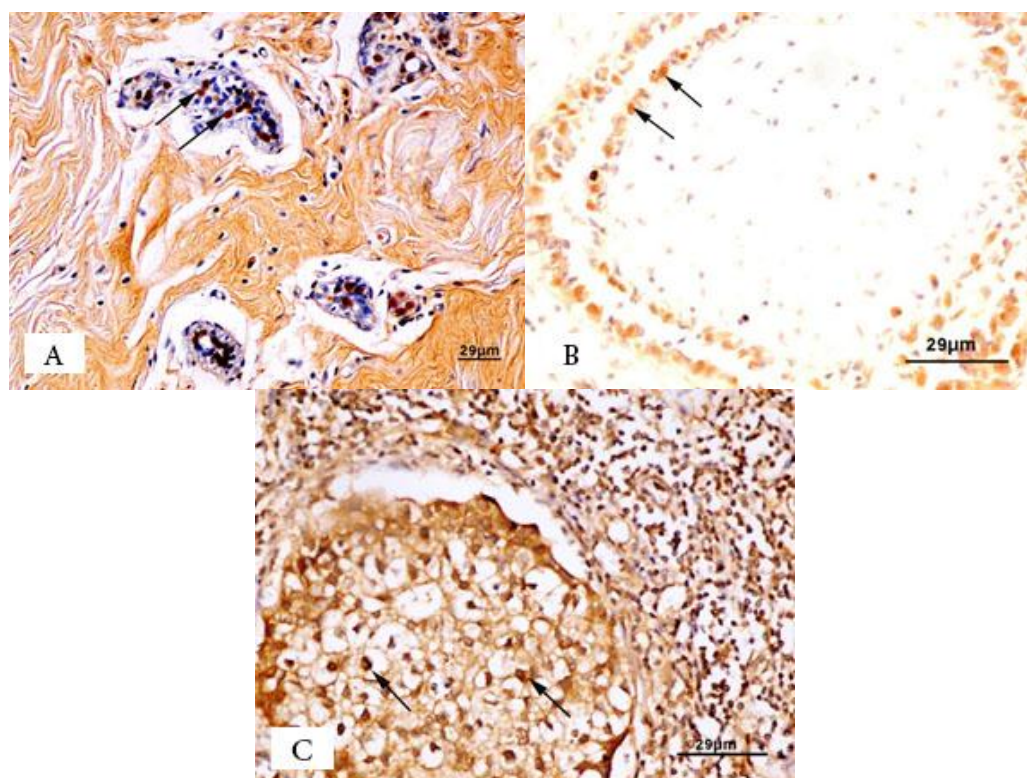

Fig. 9: Photomicrographs obtained from breast tissues (PR immunostain, counterstained with hematoxylin): A) normal breast tissue showing positive expression for PR in the nuclei of the epithelial cells (arrows), B) intracanalicular fibroadenoma showing positive expression for PR in the nuclei of the epithelial cells (arrows), C) IDC tissue showing positive expression for PR in the nuclei of the tumor cells (arrows).

DNA fragmentation results

Analysis of DNA fragmentation electrophoretic picture showed that the total optical density of released DNA was 0.6 and 0.3 in control and fibroadenoma specimens, respectively, while the total optical density in IDC specimens was statistically increased (98.2) due to fragmentation of DNA (Fig. 10). Data in table 5 showed the optical density changes in both intact and fragmented DNA in the different groups.
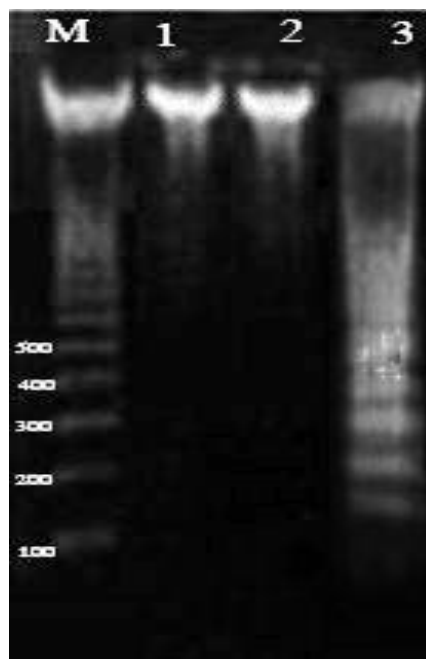

Fig. 10: Agarose gel electrophoresis of DNA extracted from breast cases. Lane M: DNA ladder100 bp (marker); Lane 1: normal breast tissue; Lane 2: Fibroadenoma and Lane 3: IDC cases.

Table 5: Changes in the total optical density of both intact and fragmented DNA in the different patient groups.

Total optical density	ladder	Control	Fibroadenoma	IDC
Intact DNA	65.19	194.09	191.45	99.8
Fragmented DNA	87.6	0.6	0.3	98.2

DISCUSSION

Breast cancer is the second most common diagnosed cancer in the world but ranks the 5th as cause of death (Ferlay *et al*., 2010).

In the present study, the mean age of female patients was 26.5 ± 8.38 for fibroadenoma group, while it was 46.17 ± 1.154 years for IDC group. Arpino *et al*. (2004) also reported that 72.2% of patients diagnosed with IDC were more than 50 years old and 27.8% of patients were less than 50 years old. Pestalozzi *et al*. (2008) found that 54.9% of IDC cases were more than 50 years old and 45% were less than 50 years. In addition, fibroadenoma was the most common breast tumor in the age group of 21–40 years (Manna *et al*., 2010).

The results showed that the tumor size of IDC cases was divided into two groups, the first group (22 cases; 55%), included tumors < 2cm while the second group (18 cases; 45%), included tumors ≥2cm. Arpino *et al*. (2004) reported that 51.3% of IDC cases had tumor size less than 2 cm while 48.6% had tumor size more than 2 cm. Pestalozzi *et al.* (2008) found that 47.9% of IDC cases ranged from 0-2 cm in size and 50.6% of cases were more than 2 cm. Out of 40 IDC cases, 37 patients were diagnosed with grade II and 3 patients with grade III and none with grade I. Also, Li *et al.* (2005) classified IDC cases into 55% grade II, 22% as grade III and 19% as grade I. Pestalozzi *et al*. (2008) reported that 43.7%, 41.7% and 12.9% of IDC cases were classified as grade II, grade III and grade I, respectively. In another study, Korhonen *et al*. (2013) counted 49% as grade II, 29% as grade I and 22% as grade III.

A reduction in the polysaccharide contents in both fibroadenoma and IDC specimens compared with normal breast tissue was observed. The IDC cases contained the lowest polysaccharide contents. Such reduction in polysaccharide contents may be due to alteration in carbohydrate metabolism. The proliferating tumor cells generate the energy that is required to support rapid cell division and must evade the checkpoint controls that would normally block proliferation under the stressful metabolic conditions that are characteristic of the abnormal tumor microenvironment (Cairns *et al*., 2011). The best explanation for this is the Warburg effect, which is a shift from adenosine triphosphate (ATP) generation through oxidative phosphorylation to ATP generation through glycolysis, even under normal oxygen concentrations (Warburg, 1956). This shift

therefore demands that tumor cells implement an abnormally high rate of glucose uptake to meet their increased energy, biosynthesis and redox needs (Cairns *et al.*, 2011).

Total proteins showed marked difference in the specimens of the three patient groups. It was high in IDC specimens, moderate in fibroadenoma and low in normal breast tissue. The observed change in the protein content may be contributed to alteration in different protein metabolism pathways. Debald *et al.* (2011) identified five proteins that were up regulated in human breast cancer tissue, but absent in the healthy and benign controls. Increased expression of Trefoil proteins, both Trefoil factor 1 and Trefoil factor 3, has been reported in ductal breast cancers (Ahmed, 2011). Alalem *et al. (2016)* found elevation in the level of total mammalian Target of Rapamycin (mTOR) protein (an important regulator of cell growth and protein synthesis) in breast cancer cells compared to their nonmalignant counterparts. The authors explain, at least in part, the high level of mTOR protein in these cells by the defective proteolysis of mTOR protein in breast cancer cells.

Darash-Yahana *et al.* (2016) observed over expression of nutrient-deprivation autophagy factor-1 (NAF-1) (is an iron sulfur protein associated with the progression of multiple cancer types) in xenograft breast cancer tumors which induced oxidative stress tolerance, leading to rapid tumor growth.

Immunohistochemical results obtained in the present work showed that out of 40 IDC cases, Bcl-2 expressed in 24 cases (60%) and Bax expressed in 16 cases (40%). Bcl-2 and Bax expression was negative in both fibroadenoma and normal breast tissue. In invasive ductal carcinoma of the breast among Tunisian patients, Bcl-2 was expressed in 41% and Bax in only 12% of the patients (Baccouche *et al.*, 2003). The expression of Bax and Bcl-2 in 50 breast cancer Egyptian female patients at Mansoura city was studied by Ali *et al.* (2006) who found that Bcl-2 and Bax expression displayed a significant relation with increasing histological grades and negative relation to stages of IDC. Jaafar *et al.* (2012) recorded that 18.9% of IDC cases showed Bax expression while 44.8% showed Bcl-2 expression.

In the current study, 18(45%) IDC samples were positive for p53 expression and 22(55%) samples were negative for p53 expression. All fibroadenoma and normal tissue samples were negative for p53 expression. Negative p53 expression was also observed by Manna *et al.* (2013) in all the cases of control and fibroadenoma except a case was positive in fibroadenoma. Recently, Sekar *et al.* (2014) found overexpression of p53 in 71.67% (43/60) of the tumors which showed statistically significant association with higher histological grade of the tumor. Andersen *et al.* (1993) suggested that abnormal p53 can be used as an independent prognostic indicator of

shortened survival and recurrence. They observed a statistical significant association between p53 alterations and tumor size, histologic and nuclear grade, DNA ploidy, mitotic rate, proliferation index, positive node status, distant metastases, and lack of estrogen receptors. Payandeh *et al.* (2015) reported that p53 was positive in 104(45%) and negative in 127(55%) of 231 cases. The present results revealed an indirect statistical significant between the expression of Bcl-2 and Bax while there was a direct statistical significant between Bcl-2 and p53 in the IDC group.

The obtained results indicated that 30% of the IDC specimens showed positive ER expression while 70% of the IDC specimens showed negative ER expression. PR expression was positive in 40% from the IDC specimens and negative in 60% of the specimens. All the fibroadenoma and normal specimens showed positive expression of both ER and PR. The expression of ER in IDC cases revealed a significant decrease compared to control group. There was no significant between ER expressions in IDC group compared to its expression in fibroadenoma group. PR expression showed significant increase in IDC cases compared to control and fibroadenoma group while PR expression showed significant decrease in fibroadenoma compared to control group. In invasive ductal carcinoma, positive expression for both ER and PR was 74% and 53%, respectively (Nadji *et al.*, 2005). Zengel *et al.* (2015) reported that in invasive ductal carcinoma, expression of ER and PR was positive in 59.9% and 60.5% and negative in 40.1% and 39.5%, respectively. Umekita *et al.* (2007) reported that the positive expression of ER and PR in breast carcinoma was 74% and 62% of the studied cases, respectively. Payandeh *et al.*(2015) observed that ER expression was positive in 135(58.4%) and negative in 96(41.6%) of the cases and PR expression was positive in 128(55.4%) and negative in 103(44.6%) of 231 cases. Significant correlation between ER and PR expression was found by Dodiya *et al.* (2013). ER and PR positivity increased with advancing age in breast carcinoma patients. The expression of hormone receptors was higher in infiltrating ductal carcinoma subtypes as compared to other subtypes of breast carcinoma. Significantly higher ER and PR levels were recorded in breast cancer cells than in normal terminal duct lobular unit (Yang *et al.*, 2013).

In agreement with the fibroadenoma observations (positive expression of both ER and PR) obtained in the present study, Khanna *et al.* (2012) observed variable positive ER expression among the studied fibroadenoma cases. Gupta *et al.* (2015) observed positive expression for both ER and PR in 24 out of 25 cases (96%) of benign breast lesions. In contrast, Branchini *et al.* (2009) observed alterations in the estrogen receptor alpha protein expression between normal breast tissue and fibroadenoma. The absence of estrogen receptor alpha protein levels could be a characteristic behavior of fibroadenomas, unlike breast cancer.

Our data showed significant indirect statistical relationship between p53 and ER or PR in the studied three groups. Negative ER and PR status was associated with p53 expression (Dookeran *et al*., 2010; Ahmed *et al*., 2011). Payandeh *et al.* (2015) observed significant correlation between the expression of p53 with ER and PR ($p<0.05$). More patients with p53-positive have age equal 50 years, higher grade, ER-negative and PR-negative. In contrast, Song *et al*. (2006) found that over expression of p53 in invasive ductal carcinoma was not related to hormone receptor status.

Analysis of DNA fragmentation electrophoretic pictures of normal, fibroadenoma and IDC cases showed that the total optical density of released DNA was 0.6 and 0.3 in control and fibroadenoma specimens, respectively, while it significantly increased (98.2) in IDC specimens indicating DNA fragmentation. Many studies confirmed DNA fragmentation in breast cancer. Bhatia *et al*. (2013) noted that gamma H2AX (H2A histone family, member X), a marker of DNA double stranded breaks) was highly expressed in IDC as compared to fibroadenoma. Amongst the IDC cases, the γH2AX was found to be significantly over expressed in DNA diploid IDC cases as compared to the aneuploid ones. Abdel-Fatah *et al*. (2014) observed low expression of DNA-dependent protein kinase catalytic subunit (a critical component of the non-homologous end-joining pathway required for the repair of DNA double-strand breaks) was associated with poor breast cancer-specific survival, higher tumor grade, higher mitotic index, tumor de-differentiation and tumor type.

A DNA ladder pattern was evident in invasive ductal carcinomas. This indicates that the cells die by apoptosis, where DNA damage is one of the features of apoptosis. One of the earliest biochemical events associated with apoptosis was the double stranded internucleosomal cleavage of DNA and this results in DNA fragments that are multiples of 180 -200 base pairs (Czene *et al*., 2002). Jänicke *et al*. (1998) investigated the requirement for caspase-3 in apoptosis, in MCF-7 breast carcinoma cell line; their results indicated that although caspase-3 is not essential for tumor necrosis factor- or staurosporine-induced apoptosis, it is required for DNA fragmentation and some of the typical morphological changes of cells undergoing apoptosis.

IN CONCLUSION
Histochemical results showed noticeable differences in polysaccharides and protein contents. Normal breast tissues contained the highest polysaccharides contents, while fibroadenoma specimens showed moderate content and the lowest content was in IDC specimens. The total proteins in invasive ductal carcinoma specimens showed significant increase compared with fibroadenoma and normal specimens. There was significant increase in Bcl-2, Bax and p53 expression in invasive ductal carcinoma compared with the other groups. On the contrary, there was a marked decrease in ER expression in both fibroadenoma and invasive ductal carcinoma compared with the normal breast. In contrast, PR expression showed significant increase in invasive ductal carcinoma group compared with normal breast group. There was high DNA fragmentation in invasive ductal carcinoma.

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