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EVALUATION OF IMMUNOHISTOCHEMICAL ANTI-APOPTOTIC BCL-2 AND PRO-APOPTOTIC BAX GENE PRODUCTS IN BREAST CARCINOMA

By

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ABSTRACT

One of the greatest challenges in breast cancer management is to accurately predict the outcome for each patient. To do this at present, we rely heavily on traditional pathological variables, such as tumor size, tumor grade and lymph node status. However, despite the broad applicability of clinic-pathological indices, they are still unable, to separate the 30% node-negative patients who will relapse from the 70% who will not.

We aimed to: 1. Study the immunohistochemical profile of bcl-2 and bax in breast carcinoma. 2. Assess their prognostic values in relations to clinico-pathological prognostic factors.

Subjects and methods: This study included 45 specimens of breast carcinoma. Patient's age, tumor size and local aggressive changes, history of recurrence and/or presence of distant metastasis were obtained. Sections stained with H&E were evaluated for the presence of benign breast disease, tumor type and tumor grade, presence of *in situ* component, lymphocytic infiltration, lymphovascular invasion and axillary lymph node status. Immunostaining was done to detect the expression of bcl-2 and bax.

Results: Both bcl-2 and bax were regularly expressed in areas of benign breast disease. Bcl-2 was positive in all ductal carcinoma *in situ* (DCIS); 100% of grade I, 89% of grade II and 87% of grade III invasive breast carcinoma (IBC). Bcl-2 was inversely correlated to tumor grade of IBC ($p<0.03$) and to lymphocytic infiltration ($p<0.02$). Bax was positive in (80%) low grade, (100%) intermediate and high grade DCIS. Bax was positive in (83%) grade I IBC and in all (100%) grade II and grade III IBC. Bax expression was positively correlated to: tumor grade ($p<0.05$) and lymphocytic infiltration ($p<0.00$). No significant difference in either bcl-2 or bax expression in lymph nodal positive or negative cases. In DCIS; negative correlation was present between bcl-2 and bax ($p<0.01$). In IBC; insignificant correlation was present between bcl-2 and bax.

Conclusions: Bcl-2 is an indicator for good prognosis in breast cancer being negatively correlated to tumor grade and prominent lymphocytic infiltration. Bax is an indicator for poor

prognosis in breast cancer being positively correlated to tumor grade and presence of prominent lymphocytic infiltration.

Abbreviations: NCI; National Cancer Institute, DCIS; ductal carcinoma *in situ*, IBC; invasive breast carcinoma, IDC NOS; infiltrating duct carcinoma, not otherwise specified, PBS; phosphate buffered solution, SI; Staining intensity, PP; percentage of positive tumor cells, IHCS; immunohistochemical scores,

INTRODUCTION

Breast cancer has emerged as a grave danger in the last 50 years, affecting as many as one in eight women during their lifetime. Recent reports have highlighted the increasing incidence of breast cancer in low- and middle -income countries. In Egypt, breast cancer is the most common cancer in women, representing 18.9% of total cancer cases in Egypt National Cancer Institute (NCI) series (Elatar, 2002). Most patients were premenopausal and relatively advanced at presentation (Omar et al., 2003).

In the last decade, basic cancer research has produced remarkable advances in our understanding of cancer biology and cancer genetics. Among the most important of these advances is the realization that apoptosis and the genes that control it have a profound effect on malignant phenotype. Changes in this cell loss factor could have a major impact on tumor growth or regression (Lowe & Lin, 2000).

Bcl-2 is a proto-oncogene that was discovered at the chromosomal breakpoint of t (14; 18) human B-cell lymphomas (Zutter et al., 1991). Bcl-2 is a major regulator of mitochondrial apoptotic events and is a member of a growing gene family consisting of two subfamilies that can inhibit (pro-survival) or can promote (pro-apoptotic) apoptosis (Tsujimoto & Shimizu**,** 2000)**.**

Anti-apoptotic proteins include: bcl -2, bcl-xl, mcl-1, bfl-1/a1, bcl-w and bcl-g. They are integral membrane proteins of the mitochondria, endoplasmic reticulum, and nuclear envelope. Pro-apoptotic proteins include: bax, bak, bok, bad, bid, bik, bim, bcl-xs, noxa, bcl-b, bcl-gl, blk and bmf. The majority of these proteins resides in the cytosol or associate with the cytoskeleton until the presence of a death signal. This causes them to integrate into the mitochondrial membrane (Simstein et al., 2003). The interplay between pro-apoptotic and pro-survival proteins plays a pivotal role in determining the eventual outcome; death or survival of the cell (Guimarães & Linden, 2004).

One of the unique features of bcl-2 family is their ability to physiologically bind each other forming a complex network of homo- and/or heterodimers. They regulate apoptosis in a rheostatic manner: in an excess of bcl-2; bcl-2/bax heterodimers are formed, which lead to inhibition of apoptosis (Reed, 1997). Conversely, in an excess of bax; for instance, bax homodimers predominate which favors apoptosis (White, 1996). Competition between family members also has an effect (Green & Evan, 2002).

In a healthy cell, the outer membranes of its mitochondria display the protein bcl-2 on their surface. Damage to the cell causes bcl-2 to activate a related protein, bax, which punches holes in the outer mitochondrial membrane, causing

cytochrome C to leak out. Activation of caspases using the energy provided by ATP occurs. This leads to digestion of structural proteins in the cytoplasm, degradation of chromosomal DNA and phagocytosis of the cell (Parton et al., 2001).

Apoptosis is often impaired in cancer and a better understanding of how bcl -2 family control apoptosis should result in new more effective therapeutic approaches (Parton et al., 2001). Bcl-2 gene is activated by chromosomal translocations in many solid tumors including breast carcinoma (Green & Evan, 2002). Bcl-2 inhibits the pro-apoptotic activity during oncogenesis, support the survival of established cancer cells, and increase resistance to chemotherapy (Khorchid & Beauparlant, 2004).

Studies suggest that bcl-2 expression is associated with enhanced response to endocrine therapy (Keen et al., 1997). Patients whose tumors co-expressed ER and bcl-2 derived the greatest benefit from hormonal therapy. This may be explained in part by the suggestion that bcl-2 is an estrogen-regulated protein (Gee et al., 1994). However, whether bcl-2 expression is an independent prognostic factor or not is a subject of controversy; some authors mention that bcl-2 has limited prognostic value (Dimitrakakis et al., 2002). Others suggest that bcl-2 is an independent predictor of breast cancer outcome, particularly in the first 5 years after diagnosis (Horita et al., 2001). More work will be required to elucidate a role for bcl-2 family members as prediction factors for response to systemic therapy (Callagy et al., 2006).

Bax (bcl-2-associated X protein), a pro-apoptotic member of bcl-2 family, inhibits the anti-apoptotic action of bcl-2 (Dejean et al., 2005). Bax, a normally cytosolic protein, translocates to mitochondria following exposure to various apoptotic stimuli or stress (Hsu & Youle, 1998). In the outer membrane, bax is thought to assume an active conformation and/or interact with an outer membrane protein to form pores, releasing cytochrome C from the intermembrane space, and subsequent caspase activation (Shimizu et al., 1999). Over -expression of bax has been shown to accelerate apoptosis in response to a variety of stimuli, including growth factor withdrawal (Brady et al., 1996).

Loss of function mutation of bax has been identified in several human tumors and knockout of bax gene results in tumorigenesis in mice, suggesting that bax acts as a classic tumor suppressor gene in vivo (Dejean et al., 2005). Bax is ubiquitously distributed in normal tissues and is regarded as a tumor suppressor sensitizing malignant cells to anticancer drugs (Godlewski et al., 2001). Bax is normally expressed in several epithelia including those in breast (Krajewski et al., 1994). In contrast to p53, bax mutation does result in loss or reduction of bax expression (De Angelis et al., 1998).

Reduced expression is associated with poor response rates to chemotherapy and shorter survival in metastatic breast adenocarcinoma (Krajewski et al., 1995). Shaw (1996) hypothesized that bax gene doesn't trigger apoptosis but simply accelerates the rate at which apoptosis proceeds.

This work aims to:

1. Study the immunohistochemical profiles of bcl-2 and bax and the potential relationships between these biological markers in breast carcinoma.
2. Assess the prognostic values of these markers and their relations with different clinico-pathological prognostic parameters of breast carcinoma.

PATIENTS AND METHODS

This study included 45 specimens of breast carcinoma; 13 prospective cases were retrieved from Department of General Surgery, and 32 retrospective specimens were retrieved from Histopathology Lab of, Sohag University Hospital, in the period from 2001-2007. Clinical data were obtained from hospital data sheets including: patient age, tumor size, evidence of axillary lymphadenopathy, presence of distant metastasis (clinical evidence and previous histopathological examination), local changes of aggressiveness, and history of recurrence. The tumor was located on the right side in 22/45 (49%) cases, on the left side in 15/45 (33%) cases, bilateral in 2/45 (5%) cases. Tumor side was unknown in 6/45 (13%) cases. Specimens included 14 modified radical mastectomies, 6 lumpectomy with axillary clearance, 12 excisional biopsies, 10 incisional biopsies and 3 simple mastectomies. Lymph node status was assessed in: 14 cases of Patey operation, 6 cases of lumpectomy and axillary clearance, and additional 5 cases in which there was strong clinical evidence of lymph node metastasis (enlarged hard fixed axillary lymph nodes).

Histopathological evaluation:

Formalin fixed, paraffin embedded five micron tissue sections were prepared, mounted on poly L lysine coated slides and dried overnight at 37°C. Sections were deparaffinized in xylene, rehydrated through graded concentrations of ethanol to distilled water and stained with H&E.

Tumors were classified into three groups according to **their size**; ≤ 2 cm, > 2–5 cm, > 5 cm according to Guerra et al. (2003). Tumors were **histopathologically** typified according to the WHO classification of breast tumors (Ellis et al., 2003). **DCIS** was classified according to the criteria of Holland et al. (1994), into: well differentiated (Grade I), intermediately differentiated (Grade II) and poorly differentiated (Grade III) DCIS. In specimens showing more than one histological grade, DCIS was graded according to the highest grade. **IBC** were classified according to the Elston and Ellis grading system (1998) into; well differentiated (Grade I), moderately differentiated (Grade II), and poorly differentiated (Grade III). **Lymph nodal status** was also classified according to the number of the affected lymph nodes into, no lymph node affection (grade 1), one to three affected nodes (grade 2), and four or more affected nodes (grade 3) after Guerra et al. (2003). **Lymphocytic infiltration** was evaluated as follows; <50% (+), equal to 50% (++), or >50% (+++) per field at low magnification (X10) after Mañes et al. (2004). **Lymphovascular invasion (LVI)** was considered evident when at least one tumor cell cluster was clearly visible inside a vascular channel lined by a single layer of endothelial cells without red blood cells (Van der Auwera et al., 2005). **Desmoplastic stroma** was considered evident when dense collagen stroma is

present with apparently few stromal cells (Walker et al., 2001).

Immunohistochemical procedures:

Immunohistochemical staining with antibodies (Abs) for bcl-2 and bax was performed using immunoperoxidase technique. Sections were pretreated by boiling for 9-15 min in citrate buffer; pH 6.0, incubated for 1-2 hours in humid chambers at room temperature with 1/100 mouse monoclonal antibodies for bcl-2 (clone 100/D5, catalogue MS123-P0, LabVision) and bax (clone 2D2, catalogue MS -771-P0, LabVision) in 1% blocking anti-goat serum. Then sections were incubated for 15 min with biotinylated secondary antibody (Ultravision detection system, anti-polyvalent, HRP, catalogue TP-060-HLX, LabVision). All dilutions were made in phosphate buffered solution (PBS), pH 7.2. Finally, the sections were lightly counterstained in Mayer's Hematoxylin and mounted on glass slides using DPX (BDH Ltd, Poole, United Kingdom).

Positive and negative controls:

A lymph node with reactive hyperplasia and normal colonic mucosa were used as positive controls for bcl-2 and bax respectively (Rehman et al., 2000). Negative controls were performed by omitting the primary antibody.

Immunohistochemical analysis of bcl-2 and bax in tissue sections:

Immunostaining of bcl-2 and bax was analyzed and evaluated in 10 different tumor fields. For either bcl-2 or bax; tumor cells are considered positive when they display a distinct micropunctate golden yellow cytoplasmic staining. Percentage of positive tumor cells (PP) was evaluated and scored as: 0 for < 5%, (1) for 5-25%, (2) for 25-50, (3) for 50-75 and 4 for >75 following. Staining intensity (SI) was considered as (1) for weak, (2) for medium and (3) for intense staining in evaluation of immunohistochemical expression of bcl-2 and bax according to Hussein et al. (2004). Immunohistochemical scores (IHCS) were calculated by multiplying PP with the SI. Hence, the following formula was used; IHCS= PP X SI. Validation of this method has been described by Damron et al. (2004).

Statistical analysis:

Chi-Square and Pearson's Correlation Coefficient tests were used to evaluate statistical significance of studied parameters as predictors for prognosis, individually and in relation to each other, with a statistical significance of $p<0.05$ for Chi-square test and $p<0.02$ for Pearson's Correlation Coefficient test (Leach, 2004).

RESULTS

Clinical findings:

The age range of the 45 studied patients was 31-87 years. Mean age was 52 years; 16 (36%) cases below or equal to the age of 50 years and 29(64%) cases above the age of 50 years. The tumor size was 2-5 cm in 21/45 (47%) cases, and > 5 cm in 24/45(53%). Local aggressive manifestations e.g. fixation, *peau d'orange*, nipple retraction, skin ulceration and fungation were present in 10/45 (22%) cases. Axillary lymph node metastasis was assessed in 25/45 (56%) of patients. They were positive in 17/25 (68%) cases, and negative in 8/25 (32%). One case had distant bone metastasis.

Histopathological findings:

Applying the WHO classification of breast tumors (Ellis et al., 2003) revealed that IDC NOS were found in 33/45(73.3%) cases. Lobular carcinoma was found in 2/45 (4.4%) cases. Medullary carcinomas were found in 3/45 (6.7%) cases. Neuroendocrine differentiation was found in 1/45(2.2%) cases. Papillary carcinomas were found in 1/45 (2.2%) case. Micropapillary carcinomas were found in 3/45(6.7%) cases. Glycogen rich carcinoma were found in 1/45 (2.2%) case. Cribifom carcinoma was found in 1/45 (2.2%) case.

Benign breast disease was found in 18/45 (40%) cases. *In situ* component was present in 16/45 (36%). DCIS was found in 13/33(39%) cases of IDC NOS; of solid, commedo, papillary, micropapillary, clinging, and cribriform patterns. *In situ* commedo, and micropapillary carcinoma was seen in 1/2 cases of micropapillary carcinoma. *In situ* cribriform carcinoma was seen in the case of invasive cribriform carcinoma. *In situ* lobular carcinoma was found in 1/2 case of lobular carcinomas. Lymphovascular invasion was present in 23/45 (51%) cases. Prominent lymphocytic infiltration was present in 5/45(11%) cases. Desmoplasia was present in 29/45 (64%) as shown in table (2).

Histopathological grading of breast carcinomas studied:

DCIS was grouped into; 5/13 (42%) low grade, 3/13 (16%) intermediate grade, and 5/13 (42%) high grade cases. IBC was grouped into; 6/45 (13%) grade I, 28/45 (62%) grade II and 11/45 (25%) grade III cases (table 1).

Immunohistopathological findings:

Bcl-2 was regularly expressed in areas of benign breast disease (18/18) of the studied cases (figure. 1). Bcl-2 was positive in all cases of DCIS with variable intensities (table 2, graph 1). Bcl-2 expression was positive in 6/6 (100%) cases of grade I (figure. 2), in 25/28 (89%) cases of grade II (figure. 3), and in 9/11 (87%) cases of grade III IBC (table 3, graph 2). Bcl-2 was significantly inversely correlated to tumor grade of IBC ($p<0.03$) and lymphocytic infiltration ($p<0.02$) as shown in table (4). No significant difference in bcl-2 expression in lymph nodal positive or negative cases (table 5)

Bax was regularly expressed in areas of benign breast disease (18/18) of studied cases. Bax was positive in 4/5 (80%) cases of low grade, 3/3 (100%) cases of intermediate grade and in 5/5 (100%) cases of high grade DCIS (table 6, graph 3). Bax was positive in 5/6 (83%) grade I, all 28/28 (100%) cases of grade II, (figure. 4 & 5), and 8/8 (100%) cases of grade III IBC (table 7, graph 4). Bax expression was positively correlated to: tumor grade ($p<0.05$) and lymphocytic infiltration ($p<0.00$). Insignificant positive correlation was found between bax expression and lymphovascular invasion ($p<0.07$) as shown in table (8). No significant difference in bax expression in lymph nodal positive or negative cases (table 9). Bcl-2 and bax expression in special types of breast cancer was shown in table (10) and figure (6). In DCIS of the breast: Negative correlation was present between bcl-2 and bax ($p<0.01$). In IBC: Insignificant correlation was present between bcl-2 and bax ($p<0.514$).

Table (1): Histopathological data of studied patients (n=45)

Parameter	**No. of cases**
Histological types	
IDC NOS	33 (73.3%)
Lobular carcinoma	2 (4.4%)
Medullary carcinoma	3 (6.7%)
Neuroendocrine carcinoma	1 (2.2%)
Micropapillary carcinoma	3 (6.7%)
Papillary carcinoma	1 (2.2%)
Glycogen rich	1 (2.2%)
Cribriform carcinoma	1 (2.2%)
Ductal carcinoma *in situ* (13)	
Low grade	5 (42%)
Intermediate grade	3 (16%)
High grade	5 (42%)
Tumor grade of IDC (45)	
Grade I	6 (13%)
Grade II	28 (62%)
Grade III	11 (25%)
Lymphovascular invasion	
Absent	22 (49%)
Present	23 (51%)
Lymphocytic infiltrate	
Minimal	40 (89%)
Prominent	5 (11%)
Desmoplasia	
Minimal	16 (36%)
Prominent	29 (64%)

Table (2): Bcl-2 expression in DCIS of the breast

Tumor grade	Bcl-2 expression (IHCS)							IHCS (X±SD)
	0	2	4	6	8	9	12	
Low grade (5)	0	0	1	0	1	0	3	9.6±3.6
Intermediate grade (3)	0	2	0	1	0	0	0	3.3±2.3
High grade (5)	0	3	1	1	0	0	0	3.2±1.8
P value	<0.1							

Table (3): Bcl-2 expression in IBC according to the grade

Tumor grade	Bcl-2 expression (IHCS)							IHCS (X±SD)
	0	2	4	6	8	9	12	
Grade I (6)	0	0	0	0	3	1	2	9.5±2.0
Grade II (28)	3	1	5	8	5	2	4	6.3±3.5
Grade III (11)	2	1	3	0	1	1	3	6.1±4.7
P value	<0.03							

Table (4): Bcl-2 expression in IBC in relation to clinic-pathological factors

Bcl-2 expression				
Clinico-pathological Parameter		**Low (IHCS≤6) (23 cases)**	**High IHCS>6 (22cases)**	**P value**
Age				
<50	16	8	8	0.9
>50	29	15	14	
Tumor size				
2-5	21	11	10	0.9
>5	24	12	12	
Tumor grade				
Grade I	6	0	6	0.03
Grade II	28	17	11	
Grade III	11	6	5	
Lymphovascular invasion				
Absent	22	10	12	0.6
Present	23	13	10	
Lymphocytic infiltration				
Minimal	40	18	22	0.02
Prominent	5	5	0	
Desmoplasia				
Absent	16	10	6	0.3
Present	29	13	16	

Table (5): Bcl-2 expression in IBC in relation to lymph node status

	Bcl-2 expression	
	Low (17)	High (8)
Negative (8)	4	4
Positive (17)	13	4
P value	<0.2	

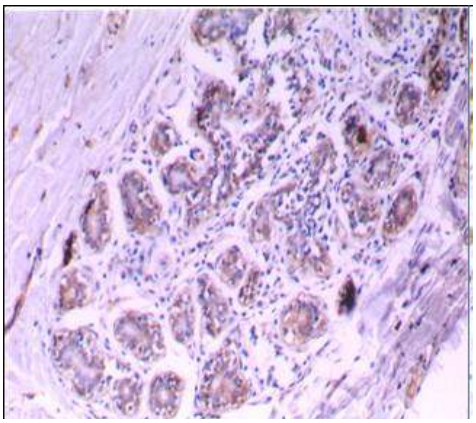


Figure (1): Area of strong bcl-2 in lobular adenosis of the breast (X100)

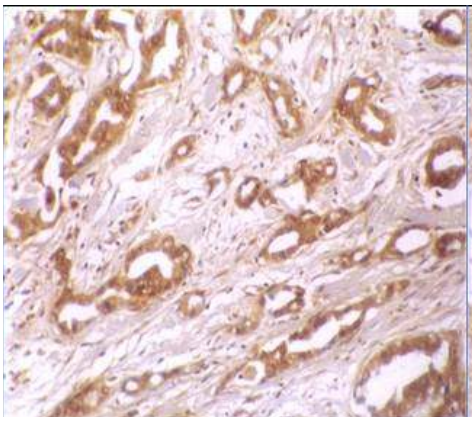


Figure (2): High bcl-2 expression in grade 1 IDC NOS (X200)

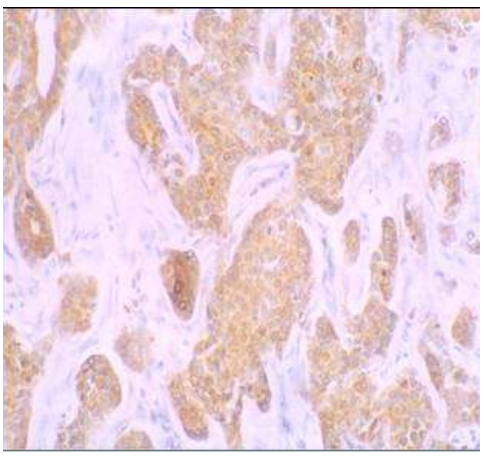
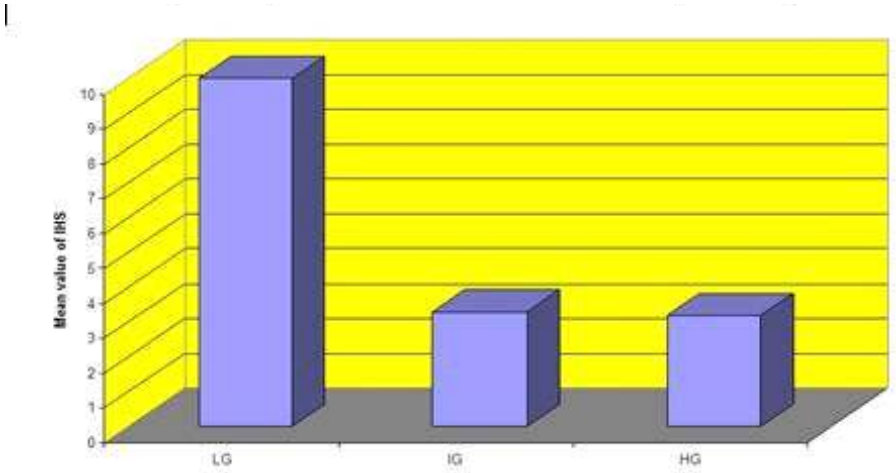
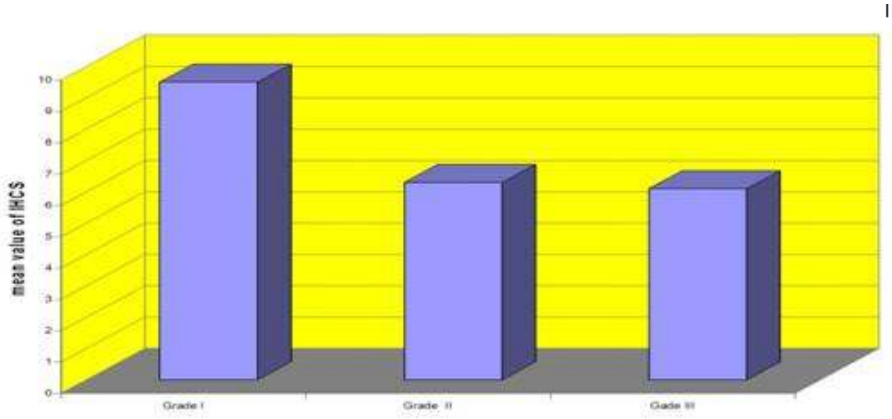


Figure (1): Moderate bcl-2 expression in grade II IDC NOS (X200)



Graph (1): Bcl-2 expression in DCIS according to the grade



Graph (1): Bcl-2 expression in IBC according to tumor grade

Table (6): Bax expression in DCIS according to tumor grade

Tumor grade	**Bax expression (IHCS)**							**IHCS (X±SD)**
	0	2	4	6	8	9	12	
Low grade (5)	1	1	1	0	0	0	2	**6±5.7**
Intermediate grade (3)	0	0	0	0	0	0	3	12±**0.0**
High grade (5)	0	0	0	0	0	1	4	11.6±**0.9**
P value	<0.2							

Table (7): Bax expression in IBC according to tumor grade

Tumor grade	Bax expression (IHCS)							IHCS (X±SD)
	0	2	4	6	8	9	12	
Grade I (6)	1	0	1	1	2	1	0	6.7±1.3
Grade II (28)	0	1	1	6	5	4	11	9.2±2.3
Grade III (11)	0	0	0	0	2	1	8	11±1.7
P value	<0.05							

Table (8): Bax expression in IBC in relation to clinic-pathological factors

Bax expression				
Clinico-pathological Parameter		**Low (IHCS≤6) (11 cases)**	**High IHCS>6 (34 cases)**	**P value**
Age				
<50	16	6	10	0.1
>50	29	5	24	
Tumor size				
2-5	21	5	16	0.9
>5	24	6	18	
Tumor grade				
Grade I	6	3	3	0.05
Grade II	28	8	20	
Grade III	11	0	11	
Lymphovascular invasion				
Absent	22	8	14	0.07
Present	23	3	20	
Lymphocytic infiltration				
Minimal	40	6	34	0.00
Prominent	5	5	0	
Desmoplasia				
Absent	16	3	13	0.7
Present	29	7	22	

Table (9): Bax expression in IBC in relation to lymph node status

Bax expression		
	Low (3)	**High (22)**
Negative (8)	1	7
Positive (17)	2	15
P value	<0.96	

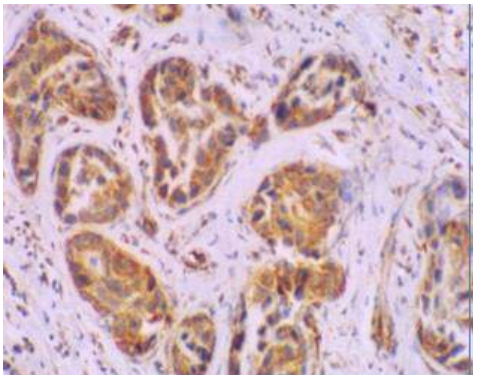


Figure (4): High bax expression in grade II IDC NOS (X200)

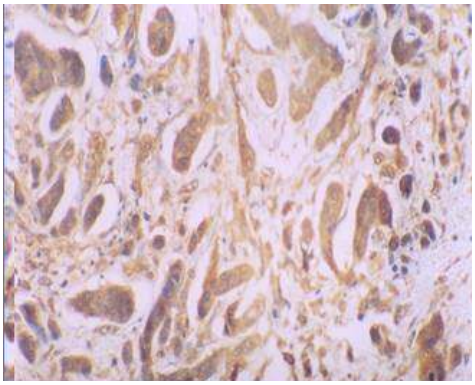


Figure (5): Area of high bax expression in grade II IDC NOS (X200)

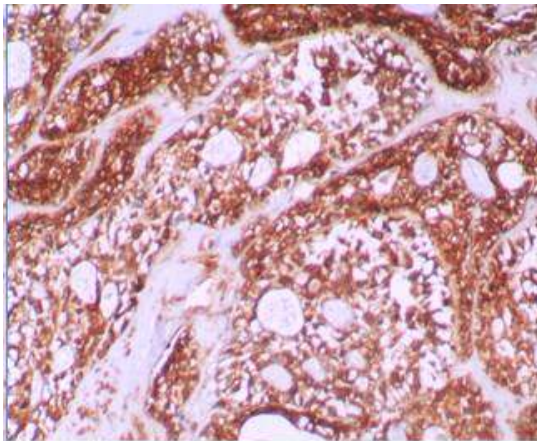
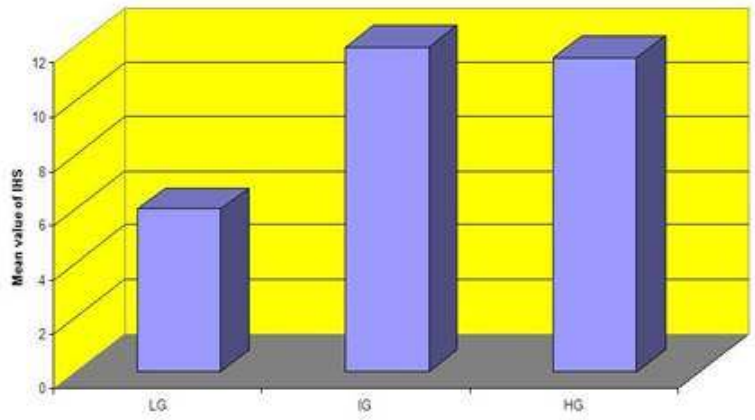
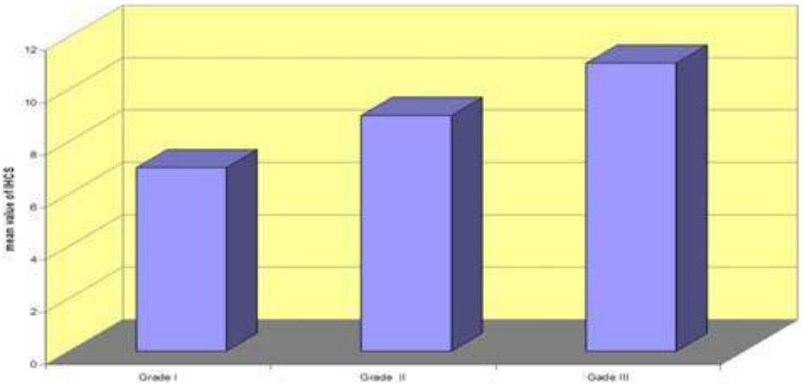


Figure (4): High bax expression in Cirbriform carcinoma of the breast (X200)



Graph (3): Bax expression in DCIS according to tumor grade



Graph (4): Bax expression in IBC according to tumor grade

Table (10): Expression of bcl-2 and bax in special types of breast carcinoma

Tumor type	Mean values of HIS		
	No.	bcl-2	bax
Lobular carcinoma	2	8	7.1
Medullary carcinoma	3	3.3	8.6
Papillary carcinoma	1	8	4
Neuro-endocrine carcinoma	1	4	4
Micropapillry carcinoma	3	3.3	10
Glycogen rich carcinoma	1	9	12
Cribriform carcinoma	1	9	12

DISCUSSION

It has been suggested that gene expression studies offer the greatest promise for refining prognostication in breast cancer (Perou et al., 2000). The current hypothesis of tumorigenesis in humans suggests that cancer cells acquire their hallmarks of malignancy through the accumulation of gene activation and inactivation events over long periods of time. For breast cancer, this multistep process may manifest itself as a sequence of pathologically defined stages. It is widely held that breast cancer initiates as the pre-malignant stage of atypical ductal hyperplasia (ADH), progresses into the pre-invasive stage of DCIS, and culminates in IDC (Ma et al., 2003).

In our study, 18/45 (40%) specimens of breast carcinoma were accompanied with benign breast disease which is a known risk factor for breast cancer. Elmore et al. (2005), stated that; for women with proliferative changes, about 10 out of 100 will develop breast cancer.

In situ component was evident only in 15/45(33.3%) cases. This percentage is much lower than the findings of Tavassoli (1999), who found foci of DCIS in up to 80% of cases of IBC. This marked difference may be explained at least in part by late discovery of cases in our locality. However, Omar et al. (2003) found even smaller percentage of carcinoma *in situ* component (1.5%), in breast cancer patients in Upper Egypt.

IDC NOS of the breast is the most commonly encountered form of IBC (Molland et al, 2004). In our series most of the specimens were IDC NOS (73.3%). This ratio looks similar to that (72.8%) found by Li et al. (2003); but slightly lower than that (88.2%) found by Saxena et al. (2005).

Tumor grade has been a highly valuable prognostic factor for breast cancer, as poorly differentiated lesions are associated with significantly poorer clinical outcome (Pages et al., 2005). In our study tumors were graded into; 6/33 (13%) grade I, 28/33 (62%) grade II, and 11/33 (25%) grade III IBC. This finding is consistent with Omar et al. (2003), who reported a low incidence of grade I tumors (5.4%) in Egyptian patients, while grades II and III tumors were 66.0% and 28.6% respectively. This could be explained by presence of certain genetic or environmental carcinogens which lead to development of aggressive tumor phenotypes in our locality.

Axillary lymph node status has repeatedly been shown to be the single most important predictor of disease-free survival and overall survival (Sebastian et al., 2002). Nodal involvement is an indicator of metastatic disease; the cause of death in patients with breast cancer (Verschraegen et al., 2005). Axillary lymph node examination is an important staging procedure for IBC (Sebastian et al., 2002). Hence; the generally admitted conclusion is that tumor size loses its prognostic role in cases of nodal involvement (Verschraegen et al., 2005). In our study axillary lymph nodes were involved in 17/25 (68%). In agreement with the findings of El-Bolkainy (2000); the frequency of axillary lymph node metastases was 75%, of Egyptian patients, and in large series studied by Jatoi et al. (1999), nodal metastasis was present in 63.3% (1,068/1,696). In contrast

Silverstein (2001); found nodal metastasis in 36% (680 /1891) of cases. These findings may indicate that incidence of lymph node metastasis is relatively lower in developed countries, which may reflect earlier detection of the tumor.

Invasion of the lymphovascular channels is a necessary gateway to the metastatic process and is an independent prognostic indicator in breast cancer (Phelan et al., 2007). In our study, lymphovascular invasion was present in 23/45 (51%) of breast cancer patients. Similar incidence (31.6%) 56/177 was found by Mohammed et al. (2007). However, other investigators found higher incidence of lymphovascular invasion in breast cancer, e.g. 78% (54/69) by Ito et al. (2007), which is most likely due to the use of lymphatic endothelial markers; D2-40 and podoplanin in the latter study. These are markers useful in accurate detection of lymphovascular invasion by tumor cells.

The degree of lymphocytic infiltration and, especially, the presence of lymphocytes within tumor cell nests have been shown to correlate with a better prognosis in many tumor types including ovarian cancer and colon cancer (Zhang et al., 2003 & Pages et al., 2005). Several studies in breast cancers support the notion that tumor infiltration by lymphocytes indicates an antitumor cellular immune response (Toso et al., 1998). Significant negative correlations were found between a reduced number of CD4+ lymphocytes or CD4+/CD8+ ratio and several histological parameters: tumor diameter, pleomorphism, and nucleus/ cytoplasm ratio. There was also a significant positive correlation between the total number of CD8+ lymphocytes infiltrating the tumor tissue and the number of axillary lymph nodes with metastatic disease. It is suggested that the reversed ratio of CD4+/CD8+ lymphocytes may significantly affect the host/tumor immune surveillance (Bilik et al., 1989). It has also been reported that the lymphocyte infiltration in HER2+ breast tumors has a preponderance of macrophages, whereas lymphocyte infiltrates in HER2− breast tumors were composed mostly of T cells (Pupa et al., 1996). Our study showed that lymphocytic infiltration was prominent in 5/45(11%) of breast cancer patient, a finding shared with Demaria et al. (2001), who found that lymphocytic infiltrate in breast carcinoma was minimal in the majority of patients.

Desmoplastic reaction is characteristic of IDC of the breast, and the intensity of this reaction can be different from case to case. The interactions between the tumor stroma and the neoplastic cells are very important, and the tumor stroma can act as a regulator of neoplastic behavior (Ferrini & Rossi, 2001). In our study, desmoplastic stroma was evident in 29/45 (64%) of IBC cases, in agreement with Ferrini & Rossi (2001), who found a ratio of 74 % of tumors with prominent desmoplasia.

Bcl-2 expression

Previous findings support the hypothesis that breast cancer evolves by clonal selection of cells that acquire multiple molecular changes through a defined progression of morphologically distinguishable stages, beginning with benign hyperplasia, which progresses to atypical hyperplasia, then to *in situ* carcinoma, and finally to invasive breast cancer (Deng et al., 1996).

Whether a cell undergoes apoptosis or survives depends on the relative expression and dimerization status of the pro-apoptotic and anti-apoptotic proteins. An increase in bcl-2 shifts the balance in favor of cell survival (Callagy et al., 2006). The opposing functions of anti- and pro-apoptotic genes arbitrate the life-or-death decision (Parton et al., 2001). However the mechanisms underlying bcl-2 expression and its significance are less certain (Callagy et al., 2006).

The information derived from bcl-2 studies will bring important insights to the physiology of both normal breast as well as breast cancer. The tumorigenic potential of bcl-2 has been shown in animal models and is supported by the finding of over-expression of bcl-2 in a variety of solid organ tumors (Pietenpol et al., 1994). In the breast, bcl-2 is expressed in normal glandular epithelium and is up-regulated by estrogen possibly as a result of direct transcriptional induction with negative regulation by p53-dependent mechanism (Haldar et al., 1994). Expression of bcl-2 was found to significantly correlate with lymphatic invasion, lymph node metastasis, but not histological differentiation, tumor grade or vascular and fatty invasion (Matsuyoshi et al., 2006). One would predict that aberrations of the bcl-2 family of proteins might be prevalent in breast cancer given that impaired apoptosis is a crucial step in neoplastic progression (Callagy et al., 2006). Bcl-2 parameter might be useful markers for cancer progression, independent of the hormone receptor status, in human breast cancers (Matsuyoshi et al., 2006).

This study revealed that bcl-2 was regularly expressed in areas of benign breast disease (18/18), which is consistent with Ferrieres et al. (1997), and confirms the major apoptosis regulatory role of bcl-2 in mammary epithelium. In areas of *in situ* carcinoma of the breast, bcl-2 expression was not correlated with lower grade DCIS, which is contradictory to Meijnen et al. (2008) who found inverse relationship between bcl-2 expression and the grade of DCIS. Our finding could be due to relatively small number (13) of areas of DCIS. In this study, bcl-2 expression was present in 39/45 (87%) of IBC. Krajewski et al. (1999),Vakkala et al. (1999) and Ioachim et al. (2000) found bcl-2 expression in IBC ratios of 80%, 70% and 40%, respectively. This difference is most likely due to variable proportions of different tumor grades in those studies.

We found highly significant negative relationship between bcl-2 expression and tumor grade in IBC ($p<0.003$). This was elucidated previously by Neri et al. (2006) and Wang et al. (2006). Moreover, Kobayashi et al. (1997) found that patients with bcl-2 expressing tumors survived without recurrence significantly more than those with tumors exhibiting reduced expression. Thus bcl-2 may be an indicator of favorable outcome in breast cancer.

Although the role of bcl-2 has been established as a key player in the control of apoptosis (Tsutsui et al., 2006), recent experimental studies have demonstrated that bcl-2 is implicated in regulating the cell cycle and proliferation. The cells over-expressing bcl-2 gene product not only showed a delayed onset of apopto-

sis but also a rapid arrest in the cell cycle, thus bcl-2 deficient cells demonstrate an accelerated cell cycle progression (Bonnefoy-Berard et al., 2004). These facts provide an explanation to why bcl-2 expression is inversely correlated to tumor grade in breast cancer (Vairo et al., 2000). Another possible explanation for this phenomenon is that bcl-2 positive tumors often have positive estrogen receptors and a more favorable prognosis. Indeed, estrogen is found to be a positive regulator of bcl-2 gene expression in breast cancer cell lines (Teixeira et al., 1995).

Being estrogen dependant (Teixeira et al., 1995), bcl-2 expression is more likely to be age dependant. However In this study we found statistically insignificant relationship between bcl-2 expression and patient's age, concurring with the findings of Poelman et al. (2000), and Quong et al. (2002). This may be due to occurrence of gene mutations, which may alter the effect of estrogen on bcl-2.

Bcl-2 was not correlated to tumor size in our study, which is consistent with the findings of Eguchi et al. (2000) and Tsutsui et al. (2006), and This finding suggests that there may be a balance between anti-apoptotic and anti-proliferative activity of bcl-2 in breast carcinoma, so that the net effect of bcl-2 on tumor size is abolished. On the other hand, Anagnostopoulos et al. (2007) found positive correlations between bcl-2 expression and smaller tumor size. This difference may be explained by variable co-expression of bcl-2 with other molecules that affect tumor size e.g p53, Ki-67, or other bcl-2 family members.

Concurring with Ioachim et al. (2000) and Tsutsui et al. (2006) we found negative correlations between bcl-2 expression and lymph node status but this difference was not statistically significant. However Coradini et al. (2002) and Neri et al. (2006) showed that bcl-2 was not correlated to the presence of lymph node metastasis in breast carcinoma. This difference may be explained by the effect of other co-expressed molecules which affect tumor metastasis, e.g p53 and bcl-xl or the small number of cases included in this study.

This study also showed that no correlation was present between bcl-2 expression and presence of vascular invasion in breast cancer, consistent with the findings of Giatromanolakia et al. (1998). However, Neri et al. (2006), found a significant negative correlation between bcl-2 and presence of vascular invasion in breast cancer. Expression of bcl-2 was found to significantly correlate with lymphatic invasion, lymph node metastasis, but not histological differentiation, tumor grade or vascular and fatty invasion. Bcl-2 parameter might be a useful markers for cancer progression, independent of the hormone receptor status, in human breast cancers (Matsuyoshi et al., 2006). This difference could be explained in the view of difficulty, and relative subjectivity of vascular invasion assessment, which is better done with the use of immunohistochemical markers of lymphatic endothelium e.g. D2-40 which is a reliable marker of lymphatic vessels and is a useful tool for lymphatic emboli identification in immune-stained sections of breast carcinomas with higher identification rates than H&E (Marinho et al., 2008).

This study showed inverse correlation between bcl-2 expression and lymphocytic infiltration in IBC ($p< 0.02$), which is in favor of the good prognostic value of bcl-2. This study showed that bcl-2 is not correlated with desmoplastic stroma in breast carcinoma. To the best of our knowledge, there are no previous reports discussing this correlation.

Bax expression

Bax is a major pro-apoptotic member of the bcl-2 family and a molecule required for apoptotic cell death (Xin & Deng et al., 2005). In this study bax was regularly expressed by normal mammary epithelium and areas of benign breast disease, which is consistent with Jun et al. (1999) and further supports the role of bcl-2 family members in mediating apoptosis in the mammary gland. In our study, bax expression was present in most of the cases of IBC (97%) which is higher than the range mentioned in literature; 75%, 5%, and 20%, found by Veronese et al. (1998), Sjostrom et al. (2002), and Redondo et al. (2003) respectively. This variability could be attributed to variability in tumor grades in different studies.

In agreement with Rehman et al. (2000), who found that Bax expression did not correlate to increasing histological grades of DCIS; we found statistically insignificant correlation between bax expression and tumor grade of DCIS ($p<0.2$). Contradictory findings were obtained by Kapucuoglu et al. (1997) who found that bax expression in DCIS, relates to more aggressive neoplasms. This controversy reflects the complexity of bax regulation and the need for more studies for this gene on both immunohistochemical and molecular levels to clarify its role (Rehman et al., 2000). Concurring with Anagnostopoulos et al. (2007), this study showed positive correlation between bax expression and tumor grade of IBC ($p<0.05$).

However Redondo et al. (2003) found that no correlation is present between bax expression and tumor grade. This difference can be explained by the concomitant expression and possible dimmerization of bax and bcl-2 (Oltvai et al., 1993), which can affect the expression of bax protein. This notion is supported by findings of Binder et al. (1996), who mentioned that there was a positive association between bax expression and histological grading, and that the correlation was most significant in cases where no concomitant bcl-2 expression could be detected.

It appears that alterations of the differential bax/bcl-2 expression pattern take part in deregulation of proliferation and loss of differentiation playing an important role in malignant progression, and is more important than expression of either protein alone (Binder et al., 1996).

Our study showed that there is no relation between bax expression and age of the patient, consistent with Redondo et al. (2003). Being pro-apoptotic, it might be expected that high bax expression may be negatively related to tumor size. However, in agreement with Kymionis et al. (2001) we found no correlation is present between bax expression and tumor size. This finding may be also explained by the presence of other anti-apoptosis genes e.g. bcl-2 and bcl-xl, which affects the rate of tumor cell proliferation and apoptosis and reflects the complexity of apoptosis regulation.

This study showed that bax expression is not correlated to lymph node status. Reviewing the literature regarding this correlation showed great controversy. While Redondo et al. (2003) found no relation between bax expression and lymph node status, others found low bax expression in breast carcinomas with lymph node metastasis (Bukholm et al., 2002). A third opinion found that high bax expression was associated with positive nodal status (Veronese et al., 1998). Thus, again we can conclude that regulation of bax gene is too complex, and might be affected by different mutations, intermediary molecules, or other genes which may affect its expression and function (Rehman et al., 2000).

In our study, insignificant positive correlation was present between bax expression and vascular invasion ($p<0.07$), this may support the poor prognostic role of bax expression in breast cancer. Insignificant correlation was present between bax expression and desmoplastic stroma in this study. To the best of our knowledge, no previous studies discussing these relations. A positive correlation was present between bax expression and lymphocytic infiltration ($p<0.00$), which indicates a possible role for bax gene in modulation of tumor immunity, and supports the poor prognostic value of bax gene.

Relation between bcl-2 and bax expression

In the current study, negative correlation between bax and bcl-2 expression was detected in DCIS ($p< 0.01$). This concurs with the findings of Kapucuoglu et al., (1997), who found that both proteins were expressed in normal and benign epithelium, but different staining patterns were observed according to the degree of differentiation of the neoplastic epithelium. In well-differentiated DCIS there was a predominance of bcl-2 protein staining. Grade II lesions co-expressed both proteins. Poorly differentiated DCIS displayed a predominantly bax protein staining pattern. Therefore, it appears that bax protein expression, especially in DCIS, relates to more aggressive neoplasms, while bcl-2 protein expression is associated with less aggressive malignant lesions.

In IBC we found that, no relation was present between bax and bcl-2 expression. This concurs with the findings of Vakkala et al. (1999). However Sjostrom et al. (2002), found a strong positive correlation between the percentages of bcl-2 and bax-immunopositive tumor cells. This great controversy reflects the complexity of apoptosis process, and indicates the presence of different gene mutations which may affects gene functions and interactions in different manners.

Infiltrating lobular carcinoma

We found 3/45 cases of ILC (6%), which lies within the range found in literature; 4.7% in the study of Bane et al. (2004), and 7.6% in the study of Li et al. (2003). ILC showed an immunoprofile different from IDC NOS. Similar to the findings of Coradini et al. (2002), bcl-2 protein positivity was found in ILC. Bax expression was reduced in ILC in agreement with Bassarova et al. (2002).

Medullary carcinoma

Medullary carcinoma is a poorly differentiated breast cancer with a high histological grade and paradoxically good prognosis (de Cremoux et al.,

1999). In the current study, three medullary carcinomas were found (6%), which is slightly higher than the ratio found by de Cremoux et al., (1999) who found typical MC of the breast in less than 5% of cases.

The three specimens of medullary carcinoma showed low expression for bcl-2 and moderate bax expression. The pattern of bcl-2 expression concurs with findings of Kajiwara (1999) who found that typical medullary breast carcinomas exhibited significantly lower bcl-2 positivity than other types of breast carcinoma. To the best of our knowledge, no previous studies for bax expression in medullary carcinoma are present. Thus the low expression of bcl-2 indicates that the immune-profile of medullary carcinoma reflects its aggressive histopathological features rather than its favorable outcome (de Cremoux et al., 1999).

REFERENCES

1. Anagnostopoulos, G.; Stefanou, D.; Arkoumani, E.; Chalkley, L.; Karagiannis, J.; Paraskeva, K.; Mathou, N.; Dellaporta, E.; Tsianos, E. and Agnantis, N. (2007): Expression of bax protein in gastric carcinomas. A clinicopathological and immunohistochemical study. Acta Gastroenterol. Belg.; 70: 285 -289.

2. Bane, A.; Tjan, S.; Parkes, R.; Andrulis, I. and O'Malley, F. (2004): Invasive lobular carcinoma: To grade or not to grade. Mod. Pathol.; 18: 621–628.

3. Bassarova, A.; Sedloev, T.; Christova, S.; Trifonov, D. and Nesland, J. (2002): Invasive lobular carcinoma in a hypoplastic breast. Ultrastruct. Pathol.; 26: 411-414.

4. Bilik, R.; Mor, C.; Hazaz, B.; Moroz, C. (1989): Characterization of T -lymphocyte subpopulations infiltrating primary breast cancer. Cancer Immunol Immunother. 28(2):143-7.

5. Binder, C.; Marx, D.; Binder, L.; Schauer, A. and Hiddemann, W. (1996): Expression of bax in relation to bcl-2 and other predictive parameters in breast cancer. Ann. Oncol.; 7: 129-133.

6. Bonnefoy-Berard, N.; Aouacheria, A.; Verschelde, C.; Quemeneur, L.; Marcais, A. and Marvel, J. (2004): Control of proliferation of bcl-2 family members. Biochim. Biophys. Acta; 1644: 159-168.

7. Brady, H.; Gl-Gomez, G.; Kirberg, J.; Berns, A. (1996): Bax alpha perturbs T cell development and affects cell cycle entry of T cells. EMBO J. 15:6991-7001.

8. Bukholm, I.; Bukholm, G. and Nesland, J. (2002): Reduced expression of both bax and bcl-2 is independently associated with lymph node metastasis in human breast carcinomas. APMIS; 110: 214-220.

9. Callagy, G.; Pharoah, P.; Pinder, S.; Hsu, F.; Nielsen, T.; Ragaz, J.; Ellis, I.; Huntsman, D. and Caldas, C. (2006): Bcl-2 is a prognostic marker in breast cancer independently of the Nottingham prognostic index . Clin. Cancer Res.; 12: 2468-2475.

10. Coradini, D.; Pellizzaro, C.; Veneroni, S.; Ventura, L. and Daidone, M. (2002): Infiltrating ductal and lobular breast carcinomas are characterised by different interrelationships among markers related to angiogenesis and hormone dependence. Br. Cancer; 87: 1105-1111.

11. Damron, T.; Mathur, S.; Horton, J.; Strauss, J.; Margulies, B.; Grant, W.; Farnum, C. and Spadaro, J. (2004): Temporal changes in PTHrP, bcl -2, bax, caspase, TGF-ß, and FGF-2 expression following growth plate irradiation with or without radioprotectant. Histochem. & Cytochem.; 52: 157-167.

12. De Angelis, P.; Stokke, T.; Thorstensen, L.; Lothe, R.; Clausen, O. (1998): Apoptosis and expression of bax, bcl-x, and bcl-2 apoptotic regulatory proteins in colorectal carcinomas, and association with p53 genotype/ phenotype. Mol. Pathol. 51:254-261.

13. de Cremoux, P.; Salomon, A.; Liva, S.; Dendale, R.; Bouchind 'home, B.; Martin, E.; Sastre-Garau, X.; Magdelenat, H.; Fourquet, A. and Soussi, T. (1999): p53 mutation as a genetic trait of typical medullary breast carcinoma. J. Natl. Cancer Inst.; 91: 641-643.

14. Dejean, L.M.; Martinez-Caballero, S.; Guo, L.; Hughes, C.; Teijido, O.; Ducret, T.; Ichas, F.; Korsmeyer, S.J.; Antonsson, B.; Jonas, E.A. and Kinnally, K.W. (2005): Oligomeric bax is a component of the putative cytochrome c Release channel MAC, mitochondrial apoptosis-induced channel. Mol. Biol. Cell;16: 2424–2432.

15. Demaria, S.; Volm, M.; Shapiro, R.; Yee, H.; Oratz, R.; Formenti, S.; Muggia, F. and Symmans, W. (2001): Development of tumor-infiltrating lymphocytes in breast cancer after neoadjuvant paclitaxel chemotherapy. Clin. Cancer Res.; 7: 3025-3030.

16. Deng, G.; Lu, Y.; Zlotnikov, G.; Thor, A. and Smith, H. (1996): Loss of heterozygosity in normal tissue adjacent to breast carcinomas. Cell; 274: 2057–2059.

17. Dimitrakakis, C.; Konstadoulakis, M.; Messaris, E.; Kymionis, G.; Karayannis, M.; Panoussopoulos, D.; Michalas, S. and Androulakis, G. (2002): Molecular markers in breast cancer: Can we use c-erbB-2, p53, bcl-2 and bax gene expression as prognostic factors?. Breast; 11: 279-285.

18. Eguchi, H.; Suga, K.; Saji, H.; Toi, M.; Nakachi, K.; Hayashi, S.; Azambuja, M.; Cardoso, F.; Sotiriou, C.; Larsimont, D. and Piccart, M. (2000): Different expression patterns of Bcl-2 family genes in breast cancer by estrogen receptor status with special reference to pro-apoptotic Bak gene. Cell death & Diff.; 7: 439-446.

19. Elatar, I. (2002): Cancer registration, Egypt, Cairo, National Cancer Institute.

20. El-Bolkainy, M. (2000): Topographic pathology of cancer, 2nd ed. Cairo, National Cancer Institute, Cairo University; 87.

21. Ellis, I.O.; Schnitt, S.J.; Sastre-Garau, X.; Bussolati, G.; Tavassoli, F.A.; Eusebi, V.; Peterse, J.L.; Mukai, K.; Tabar, L.; Jacquemier, J.; Cornelisse, C.J.; Sasco, A.J.; Kaaks, R.; Pisani, P.; Goldgar, D.E.; Devilee, P.; Cleton-Jansen, M.J.; Bmresen-Dale, A.; van't Veer, L. and Sapino, A. (2003): Tumors of the beast. In Tumors of the breast and female genital organs. IARC. Lyon. 11-106.

22. Elmore, J. and Gigerenzer, G. (2005): Benign breast disease -The risks of communicating risk. NEJM; 353: 297 -299.

23. Elston, C. and Ellis, I. (1998): The Breast. In Systemic pathology (3rd ed.).

Churchill Livingstone: Edinburgh. 769-897.

24. Ferrieres, G.; Cuny, M.; Simony-Lafontaine, J.; Jacquemier, J.; Roulearu, C.; Guilleux, F.; Grenier, J.; Rouanet, P.; Pujol, H.; Jeanteur, P. and Escot, C. (1997): Variation of bcl-2 expression in breast ducts and lobules in relation to plasma progesterone levels: Overexpression and absence of variation in fibroadenomas. J. Pathol.; 183: 204-211.

25. Ferrini, F. and Rossi, M. (2001): Schirrous invasive ductal carcinoma of the breast over express p53 oncoprotein: Sao Paulo Med. J.; 119: 4-6.

26. Gee, J.M.W.; Robertson, J.F.R.; Ellis, I.O.; Willsher, P.; McClelland, R.A.; Hoyle, H.B.; Kyme, S.R.; Finlay, P.; Blamey, R.W. and Nicholson, R.I. (1994): Immunocytochemical localization of bcl-2 protein in human breast cancers and its relationship to a series of prognostic markers and response to endocrine therapy. Intl. J. Cancer; 59: 619-628.

27. Giatromanolaki, A.; Sivridis, E.; Kkoukourakis, M.; Harris, A. and Gatter, K. (1998): Bcl-2 and p53 expression in stage I endometrial carcinoma. Anticancer res.; 18: 3689-3693.

28. Godlewski, M.; Motyl, M.; Gajkowska, B.; Wareski, P.; Koronkiewicz, M.; Motyl, T. (2001): Subcellular redistribution of bax during apoptosis induced by anticancer drugs. Anticancer Drugs12:607-617.

29. Green, D. and Evan, G. (2002): A matter of life and death. Cancer Cell; 1: 19–30.

30. Guerra, I.; Algorta, J.; de Otazu, D.; Pelayo, A. and Fariña, J. (2003): Immunohistochemical prognostic index for breast cancer in young women. Mol. Pathol.; 56: 323–327.

31. Guimarães, C. and Linden, R. (2004): Programmed cell death. Apoptosis and alternative death styles. Eur. J. Biochem.; 271: 1638–1650.

32. Haldar, S.; Negrini, M. and Monne, M. (1994): Down-regulation of bcl-2 by p53 in breast cancer cells. Cancer Res.; 54: 2095-2097.

33. Holland, R.; Peterse, J. and Millis, R. (1994): Ductal carcinoma *in situ*: A proposal for a new classification. Semin. Diagn. Pathol.; 11: 167-180.

34. Horita, K.; Yamaguchi, A.; Hirose, K.; Ishida, M.; Noriki, S.; Imamura, Y. and Fukuda, M. (2001): Prognostic factors affecting disease-free survival rate following surgical resection of primary breast cancer. Eur. J. Histochem.; 45: 73-84.

35. Hsu, Y.T. and Youle, R.J. (1998): Bax in murine thymus is a soluble monomeric protein that displays differential detergent-induced conformations. J. Biol. Chem. 273:10777-10783.

36. Hussein, M.; Al-Badaiwy, Z. and Georgis, M. (2004): Analysis of p53 and bcl-2 protein expression in the non-tumorigenic, pretumourigenic and tumorigenic keratinocytic hyperproliferative lesions. J. Cut. Pathol.; 244: 1-9.

37. Ioachim, E.; Malamou-Mitsi, V.; Kamina, S.; Goussia, A. and Agnantis, N. (2000): Immunohistochemical expression of bcl-2 protein in breast lesions: correlation with bax, p53, Rb, C-erbB-2, EGFR and proliferation indices. Anticancer Res., 20: 4221-4225.

38. Ito, M.; Moriya, T.; Ishida, T.; Usami, S.; Kasajima, A.; Sasano, H. and Ohuchi, N. (2007): Significance of pathological evaluation for lymphatic vessel invasion in invasive breast cancer. Br. Cancer; 14: 381-387.

39. Jatoi, I.; Hilsenbeck, S.; Clark, J. and Osborne, C. (1999): Significance of axillary lymph node metastasis in primary breast cancer. J. Clin. Oncol.; 17: 2334-2336.

40. Jun, Z.; Zhen-xiang, Y. and Jing, Z. (1999): The expression and clinical value of apoptosis control gene bcl-2 and bax in breast cancer. Chin. J. Cancer Res.; 11: 112-123.

41. Kajiwara, M.; Toyoshima, S.; Yao, T.; Tanaka, M. and Tsuneyoshi, M. (1999): Apoptosis and cell proliferation in medullary carcinoma of the breast: a comparative study between medullary and non-medullary carcinoma using the TUNEL method and immunohistochemistry. J Surg Oncol.; 70(4): 209-216.

42. Kapucuoglu, N.; Losi, L. and Eusebi, V. (1997): Immunohistochemical localization of bcl-2 and bax proteins in *in situ* and invasive duct breast carcinomas. Virchows Archiv; 430: 17-22.

43. Keen, J.C.; Dixon, J.M.; Miller, E.P.; Cameron, D.A.; Chetty, U.; Hanby, A.; Bellamy, C. and Miller, W.R. (1997): The expression of Ki-S1 and bcl -2 and the response to primary tamoxifen therapy in elderly patients with breast cancer. Br. Cancer Res. Treat.; 44: 123-133.

44. Khorchid, A. and Beauparlant, P. (2004): Bcl-2-targeted cancer therapeutics. Expert Opin. Ther. Pat.; 14: 805-818.

45. Kobayashi, S.; Hirotaka Iwase, H.; Yukashi Ito, Y.; Yamashita, H.; Iwata, H.; Yamashita, T.; Ito, K.; Toyama, T.; Nakamura, T. and Masaoka, A. (1997): Clinical significance of bcl-2 gene expression in human breast cancer tissues Breast. Cancer Res. Treat.; 42: 173-181.

46. Krajewski, S.; Blomqvist, C.; Franssila, K.; Krajewska, M.; Wasenius, V.M.; Niskanen, E.; Nordling, S.; Reed, J.C. (1995): Reduced expression of proapoptotic gene Bax is associated with poor response rates to combination chemotherapy and shorter survival in women with metastatic breast adenocarcinoma. Cancer Res 55:4471-4478.

47. Krajewski, S.; Krajewska, M.; Shabaik, A.; Miyashita, T.; Wang, H.G.; Reed, J.C. (1994): Immunohistochemical determination of in vivo distribution of Bax, a dominant inhibitor of Bcl-2. Am J Pathol; 145:1323-1336.

48. Krajewski, S.; Krajewska, M.; Turner, B.C.; Pratt, C.; Howard, B.; Zapata1, J.M.; Frenke, V.; Robertson, S.; Ionov, Y.; Yamamoto, H.; Perucho, M.; Takayama, S. and Reed, J.C. (1999): Prognostic significance of apoptosis regulators in breast cancer. Endocr. Relat. Cancer; 6: 29-40.

49. Kymionis, G.; Dimitrakakis, C.; Konstadoulakis, M.; Arzimanoglou, I.; Leandros, E.; Chalkiadakis, G.; Keramopoulos, A. and Michalas, S. (2001): Can expression of apoptosis genes, bcl-2 and bax, predict survival and responsiveness to chemotherapy in node-negative breast cancer patients?. J. Surg. Res.; 99: 161-8.

50. Leach, R. (2004): Principles of measurements research and statistics. In

The Chiropractic Theories: A Textbook of Scientific Research. 4th Ed. Baltimore: Lippincott, Williams & Wilkins. 76.

51. Li, C.; Anderson, B.; Daling, J. and Moe, R. (2003): Trends in incidence rates of invasive lobular and ductal breast carcinoma. JAMA; 289: 1421-1424.

52. Lowe, S. and Lin, A. (2000): Apoptosis in cancer. Carcinogenesis; 21: 485-495.

53. Ma, Z.; Salunga, R. and Tuggle, J. (2003): Gene expression profiles of human breast cancer progression. Gene expression profiles of human breast cancer progression. *PNAS;* 100: 5974-5979.

54. Mañes, S.; Mira, E.; Colomer, R.; Montero, S.; Real, L.; Gómez-Moutón, C.; Jiménez-Baranda, S.; Garzón, A.; Lacalle, R.; Harshman, K.; Ruíz, A.; and Carlos Martínez, A. (2003): CCR5 expression influences the progression of human breast cancer in a p53-dependent manner. Exper. Med.; 198: 1381-1389.

55. Marinho, V.; Metze, K.; Sanches, F.; Rocha, G. and Gobbi, H. (2008): Lymph vascular invasion in invasive mammary carcinomas identified by the endothelial lymphatic marker D2-40 is associated with other indicators of poor prognosis. BMC Cancer; 8: 64.

56. Matsuyoshi, S.; Shimada, K.; Nakamura, M.; Ishida, E.; Konishi, N. (2006): Bcl-2 phosphorylation has pathological significance in human breast cancer. Pathobiology. 73(4):205-212.

57. Meijnen, P.; Peterse, J.; Antonini, N.; Rutgers, E. and van de Vijver, M. (2008): Immunohistochemical categorisation of ductal carcinoma *in situ* of the breast. Br. J. Cancer; 15; 98: 137-142.

58. Mohammed, R.; Martin, S.; Gill, M.; Green, A.; Paish, E. and Ellis, I. (2007): Improved methods of detection of lymphovascular invasion demonstrate that it is the predominant method of vascular invasion in breast cancer and has important clinical consequences. Am. J. Surg. Pathol.; 31: 1825-33.

59. Molland, J.G.; Donnellan, M.; Janu, N.C.; Carmalt, H.L.; Kennedy, C.W.; Gillett, D.J. (2004): Infiltrating lobular carcinoma--a comparison of diagnosis, management and outcome with infiltrating duct carcinoma. Breast 13 (5):389-96.

60. Neri, A.; Marrelli, D.; Roviello, F.; De Marco, G.; Mariani, F.; De Stefano, A.; Megha, A.; Caruso, S.; Corso, G.; Cioppa, T. and Pinto, E. (2006): Bcl-2 expression correlates with lymphovascular invasion and long-term prognosis in breast cancer. Br. Cancer Res. Treat.; 99: 77-83.

61. Oltvai, Z.; Milliman, C.; Korsmeyer, S. (1993): Bcl-2 heterodimerizes in vivo with a conserved homolog, bax, that accelerates programmed cell death. Cell; 74: 609-619.

62. Omar, S.; Khaled, H.; Gaafar, R.; Zekry, A.R.; Eissa, S. and El-Khatib, O. (2003): Breast cancer in Egypt: A review of disease presentation and detection strategies. La Revue de Santé de la Méditerranée orientale: 9; 1-16.

63. Pages, F.; Berger, A.; Camus, M.; Sanchez-Cabo, F.; Costes, A.; Molidor, R.; Mlecnik, B.; Kirilovsky, A.; Nilsson, M.; Damotte, D.; Meatchi, T.; Bruneval, P.; Cugnenc, P.H.; Trajano-

ski, Z.; Fridman, W.H.; Galon, J. (2005): Effector memory T cells, early metastasis, and survival in colorectal cancer. N Engl J Med; 353: 2654–66.

64. Parton, M.; Dowsett, M. and Smith, I. (2001): Clinical review Studies of apoptosis in breast cancer. BMJ; 322: 1528-1532.

65. Perou, C.; Sorlie, T. and Eisen, M. (2000): Molecular portraits of human breast tumors. Nature; 406: 747–752.

66. Phelan, S.; O'Doherty, A.; Hill, A.; and Quinn, C. (2007): Epithelial displacement during breast needle core biopsy causes diagnostic difficulties in subsequent surgical excision specimens. J. Clin. Pathol.; 60: 373-376.

67. Pietenpol, J.; Papadopoulos, N.; Markowitz, S.; Willson, J.; Kinzler, K. and Vogelstein, B. (1994): Paradoxical inhibition of solid tumor cell growth by bcl-2. Cancer Res; 54: 3714–3717.

68. Poelman, S.; Adeyanju, M.; Robertson, M.; Recant, W.; Karrison, T.; Fleming, G.; Olopade, O. and Conzen, S. (2000): Human breast cancer susceptibility to paclitaxel therapy is independent of bcl-2 expression. Clin. Cancer Res.; 6: 4043-4048.

69. Pupa, S.; Bufalino, R.; Invernizzi, A.; Andreola, S.; Rilke, F.; Lombardi, L.; Colnaghi, M.I.; MÃ©nard, S. (1996): Macrophage infiltrate and prognosis in c-erbB-2–overexpressing breast carcinomas. J Clin Oncol; 14: 85–94.

70. Quong, J.; Eppenberger-Castori, S.; Moore, I.; Scott, G.; Birrer, M.; Kueng, W.; Eppenberger, U. and Benz, C. (2002): Age-dependent changes in breast cancer hormone receptors and oxidant stress markers. Br. Cancer Res.Treat.; 76: 221-236.

71. Redondo, M.; Garcia, J.; Rodrigo, I.; Villar, E.; González, C. and Morell M (2003): Expression of bax and p53 proteins in the tumourigenesis and progression of breast carcinomas. Tumour Biol.; 24: 23-31.

72. Reed, J. (1997): Double identity for proteins of the bcl-2 family. Nature 387:773-776.

73. Rehman, S.; Crow, J. and Revell, P. (2000): Bax protein expression in DCIS of the breast in relation to invasive ductal carcinoma and other molecular markers. Pathol. Oncol. Res.; 6: 256-263.

74. Saxena, S.; Rekhi, B.; Bansal, A.; Bagga, A. and Murthy, N. (2005): Clinico-morphological patterns of breast cancer including family history in a New Delhi hospital, India. A cross-sectional study. Oncol.; 3: 67-72.

75. Sebastian, M.; Oleksiy, T. and Sigrid, R. (2002): The flow cytometric DNA index can predict the presence of lymph node metastases in invasive ductal breast carcinoma. APMIS; 110: 580-586.

76. Shaw, P. (1996): The role of p53 in cell cycle regulation. Pathol. Res. Pract. 192:669-675.

77. Shimizu, S.; Narita, M.; Tsujimoto, Y. (1999): Bcl-2 family proteins regulate the release of apoptogenic cytochrome c by the mitochondrial channel VDAC. Nature 399:483-487.

78. Silverstein, M.; Skinner, K. and Lomis, T. (2001): Predicting axillary nodal positivity in 2282 patients with

breast carcinoma. World J. Surg.; 25: 767-772.

79. Simstein, R.; Burow, M.; Parker, A.; Weldon, C. and Beckman, B. (2003): Apoptosis, chemoresistance, and breast Cancer: Insights from the MCF-7 cell model system. Exper. Biol. Med.; 228: 995-1003.

80. Sjöström, J.; Blomqvist, C.; von Boguslawski, K.; Bengtsson, N.; Mjaaland, I.; Malmström, P.; Ostenstadt, B.; Wist, E.; Valvere, V.; Takayama, S.; Reed, J. and Saksela, E. (2002): The predictive value of bcl-2, bax, bcl-xL, bag-1, fas, and fasL for chemotherapy response in advanced breast cancer. Clin. Cancer Res.; 8: 811-816.

81. Tavassoli, F. (1999): Pathology of the Breast. 2nd ed. Norwalk, CT, Appleton -Lange, Stanford. 226–259.

82. Teixeira, C.; Reed, J. and Pratt, M. (1995): Estrogen promotes chemotherapeutic drug resistance by a mechanisms involving bcl-2 proto-oncogene expression in human breast cancer cells. Cancer Res.; 55: 3902-3907.

83. Toso, J.; Oei, C.; Oshidari, F.; Tartaglia, J.; Paoletti, E.; Lyerly, H.; Talib, S.; Weinhold, K. (1996): MAGE -1-specific precursor cytotoxic T-lymphocytes present among tumor-infiltrating lymphocytes from a patient with breast cancer: characterization and antigen-specific activation. Cancer Res., 56: 16-20.

84. Tsujimoto, Y. and Shimizu, S. (2000): Bcl-2 family: life-or-death switch. FEBS Lett. 466:6-10.

85. Tsutsui, S.; Yasuda, K.; Suzuki, K.; Takeuchi, H.; Nishizaki, T.; HigashI, H. and Era, S. (2006): Bcl-2 protein expression is associated with p27 and p53 protein expressions and MIB-1 counts in breast cancer. BMC Cancer; 6: 187: 1471-2407.

86. Vairo, G.; Soos, T.; Upton, T.; Zalvide, J.; DeCaprio, J.; Ewen, M.; Koff, A. and Adams, J. (2000): Bcl-2 retards cell cycle entry through $p27^{Kip1}$, pRB relative p130, and altered E2F regulation. Mol. Cell Biol.; 20: 4745-4753.

87. Vakkala, M.; Lähteenmäki, K.; Raunio, H.; Pääkkö, P. and Soini, Y. (1999): Apoptosis during Breast Carcinoma Progression. Clin. Cancer; 5: 319-324.

88. Van der Auwera, I.; Van den Eynden, G.; Colpaert, C.; Van Laere, S.; van Dam, P.; Van Marck, E.; Dirix, L. and Vermeulen, P. (2005): Tumor lymphangiogenesis in inflammatory breast carcinoma: A histomorphometric study. Clin. Cancer Res.; 11: 7637-7642.

89. Veronese, S.; Mauri, F.; Caffo, O.; Scaioli, M.; Aldovini, D.; Perrone, G.; Galligioni, E.; Doglioni, C.; Palma, P.; and Barbareschi, M. (1998): Bax immunohistochemical expression in breast carcinoma: A study with long term follow-up. Pred. Oncol.; 79: 13-18.

90. Verschraegen, C.; Vinh-Hung, V.; Cserni, G.; Gordon, R.; Royce, M.; Vlastos, G.; Tai, P. and Storme, G. (2005): Modeling the effect of tumor size in early breast cancer. Ann Surg.; 241: 309–318.

91. Walker, R. (2001): The complexities of breast cancer desmoplasia. Breast Cancer Res.; 3: 143-145.

92. Wang. Z.; Zhao, P.; Liu, M. and

Li, X. (2006): Expression of the genes FHIT, bcl-2 and bax in breast infiltrating ductal carcinoma and clinicopathological significance. Zhonghua Yi Xue Za Zhi.; 22; 86: 2171-2176.

93. White, E. (1996): Life, death and the pursuit of apoptsis. Gene Dev. 10:1-15.

94. Xin, M. and Deng, X. (2005): Nicotine inactivation of the pro-apoptotic function of bax through phosphorylation. J. Biol. Chem.; 280: 10781-10789.

95. Zhang, L.; Conejo-Garcia, J.; Katsaros, D.; Gimotty, P.A.; Massobrio, M.; Regnani, G.; Makrigiannakis, A.; Gray, H.; Schlienger, K.; Liebman, M.N.; Rubin, S.C.; Coukos, G. (2003): Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med; 348: 203–13.

96. Zutter, M.; Hockenbery, D.; Silverman, G. and Korsmeyer, S. (1991): Immuno-localization of bcl-2 protein within hematopoietic neoplasms. Blood

التقييم المناعي الهستوكيميائي لمنتجات جينات مضاد الموت المبرمج للخلايا بي سي إل-٢ ومحفز الموت المبرمج للخلايا باكس في سرطان الثدي

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الخلاصة:

واحدة من أكبر التحديات في التعامل مع سرطان الثدي هو التنبؤ بدقة النتيجة لكل مريض. وللقيام بذلك في الوقت الحاضر، فنحن نعتمد اعتمادا كبيرا على المتغيرات الباثولوجية التقليدية، مثل حجم الورم، درجة الورم، ووضع الغدد الليمفاوية. ومع ذلك، على الرغم من الشيوع الواسع لتطبيق المؤشرات الاكلينكية الباثولوجية، فإنها لا تزال غير قادرة على فصل ال ٣٠٪ من المرضى سلبية الغدد الليمفاوية الذين سوف يعانون من الانتكاس من ال٧٠٪ من المرضى الذين لن يعانوا.

وقد هدفنا هنا الى: دراسة التعبير المناعى الهستوكيميائئ للبروتين بي سي ال-٢ وباكس في سرطان الثدي. وتقييم قيمها النذيرية وعلاقتها بالعوامل النذيرية الاكلينكية الباثولوجية.

المرضى والطرق المستخدمة: وقد شملت هذه الدراسة ٤٥عينة من سرطان الثدي، وقد تم الحصول على عمر المريض وحجم الورم والتغيرات الموضعية العدوانية، وتاريخ وجود تكرار أوانبثاث للورم بعيدا. وجرى تقييم المقاطع المصبوغة بالهيماتوكسيلين والايوسين لوجود أمراض الثدي الحميدة، ونوع الورم، ودرجة الورم، ووجود الورم في الموقع، والارتشاح الليمفاوي، وغزو الاوعية الدموية الليمفاوية، ووضع الغدد الليمفاوية في الإبطين. وقد تم عمل الصبغة المناعية الهستوكيميائية للكشف عن تعبيري ال بي سي ال-٢ و ال باكس.

النتائج: وقد أظهرت النتائج إنتظام تعبير كل من بي سي ال-٢ وباكس في أمراض الثدي الحميدة. وقد أظهرت النتائج ايجابية ال بي سي ال-٢ في جميع سرطانات القنوات في

الموقع، وكل عينات الدرجة الاولى و ٨٩٪ من عينات الدرجة الثانية ٨٧٪ من عينات الدرجة الثالثة من سرطانات الثدي الغازية. وقد ارتبط تعبير ال بي سي ال-٢ عكسيا مع درجة الورم ومع الارتشاح الليمفاوي. وكان تعبير باكس إيجابيا في ٨٠٪ من عينات الدرجة الأولى وكل عينات الدرجة الثانية والثالثة من سرطان القنوات في الموقع، وفي ٨٣٪ من عينات الدرجة الأولى وكل عينات الدرجة الثانية والثالثة من سرطان الثدي الغازي. وقد ارتبط تعبير باكس إيجابيا مع درجة الورم ومع الارتشاح الليمفاوي. ولم يكن هناك علاقة إحصائية بين أي من تعبيري بي سي ال-٢ أو باكس وايجابية او سلبية الغدد الليمفاوية للورم. وفي سرطانات القنوات في الموقع وجدت علاقة احصائية سلبية بين كل من بي سي ال-٢ وباكس، أما في سرطانات الثدي الغازية فلم توجد أي علاقة إحصائية بين كل من بي سي ال-٢ وباكس.

الاستنتاجات: يعتبر تعبير بي سي إل-٢ مؤشرا ذو دلالة جيدة في تشخيص سرطان الثدي في كونه يرتبط سلبا مع درجة الورم وزيادة الارتشاح الليمفاوي. اما تعبير باكس فيعد مؤشرا ذو دلالة سلبية في تشخيص سرطان الثدي في كونه يرتبط إيجابيا مع درجة الورم ووزيادة الارتشاح الليمفاوي.

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