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Evaluation the role of some biomarkers in Egyptian women with breast cancer

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Abstract: Breast cancer (BC) is the primary prevalent malignancy among women globally. Currently, it is now recognized that evasion of cell death is a hallmark of cancer. Bcl-2 gene is involved in the mechanism of cell survival and prolongs cell life by preventing apoptosis through activation of various signaling pathways. **Aim:** We aimed to evaluate whether Bcl-2 is correlated with BC among Egyptian women, and its association with clinical and pathological characteristics of the disease. **Method:** This study was designed to include 200 women including 100 breast cancer patients and 100 unrelated women without cancer . Blood samples were aspirated from all the participants and serum CA15.3, CEA and Bcl-2 levels were measured using ELISA technique. **Results:** The results obtained displayed that the serum levels of CA15.3, CEA and Bcl-2 were significantly associated with an increased risk of breast cancer compared to the healthy group ($P < 0.001$ for all). **Conclusions:** The serum levels of CA15.3, CEA and Bcl-2 are correlated with an breast carcinoma among Egyptian women.

keywords: Breast cancer, biomarkers, Bcl2, CEA, CA15.3.

1.Introduction

The most prevalent cancer to be diagnosed in women is breast cancer, which is on the rise globally (1). Due to a number of carcinogenic elements, BC is a malignant tumor that poses a severe threat to the lives and health of women worldwide. About 25% of female cancers are caused by it, and its incidence is rising annually. It also tends to be younger (2). Breast cancer is a very diverse tumor, and it has progressed through a number of intricate biological processes, including several genes and stages (3). Furthermore, Sun and Zhao discovered that the development of BC is linked to both the prevention of apoptosis and the mutation, deletion, or activation of certain proto-oncogenes and tumor suppressor genes. In actuality, the development of breast cancer and the proliferation of breast cancer cells are intimately related to the BCL-2 gene, which is implicated in cell death (4).

The BCL-2 gene can delay the growth of cancer cells by prolonging the cell cycle and inhibiting apoptosis. Therefore, breast cancer may develop when the BCL-2 gene's expression is reduced (5). One of the molecular biomarkers to predict the development of lymph node metastases in cancer patients is the relatively excellent pathophysiological behavior of patients with high expression of the BCL-2 gene (6). Apoptosis is responsible for the deliberate culling of cells throughout the process of maintaining normal development (7). Apoptotic clearance of cells is a physiological process that is usually regarded as an essential mechanism for controlling death. It happens during normal cell development and in injury (8). The external and endogenous pathways are the two main apoptotic pathways that scientists have discovered thus far, and both aim to cause cell death (9).

The endogenous (mitochondrial) pathway, is activated by the intrinsic mechanisms of the cell itself, whereas the exogenous pathway, is triggered by pro-apoptotic stimuli external to

the cell. Cancer can develop as a result of evading apoptosis, which is essential to the pathophysiology of cancer (9). Important regulators with pro- and anti-apoptotic properties include members of the B-cell lymphoma 2 (BCL-2) protein families. Healthy cells maintain a delicate equilibrium between these regulators. In fact, they have the ability to either permanently prevent cells from undergoing apoptosis and become malignant clones, or they can induce cells to irreversibly go toward cell death (10).

The proteins of the BCL-2 family are categorized into three subgroups based on the presence of short conserved sequence regions (BCL-2 homology [BH] motifs). These subgroups include the pro-apoptotic proteins (BAX and Bak), the anti-apoptotic/pro-survival proteins (BCL-2 and BCL-XL), and the pro-apoptotic BH3-only proteins (BAD and BID) (11). BCL-2 is an anti-apoptotic protein which inhibit apoptosis by forming a heterodimer with BAX and, it can also inhibit caspases-9, 3, 6, and 7 activities (12). Apoptosis inhibition prolongs the life of cancer cells and causing the cells to transform into malignant cells (13). However, we intended to evaluate whether Bcl-2 is correlated with breast cancer in Egyptian women, and its association with different clinical and pathological characteristics of the disease.

2. Materials and methods

Study population

Two hundred people participated in this case-control study, 100 of whom were BC women and 100 of whom were unrelated cancer-free controls. They were matched in age and gender, from a similar socioeconomic background and geographic location. Between April 2023 and May 2024, cases were gathered from the Medical Oncology Center's outpatient clinic at Mansoura University in Egypt. As the gold standard for identifying breast cancer, oncologists used histology and fine needle aspiration biopsies to diagnose patients (14).

Exclusions were made for breast cancer patients having a history of tumors, chemotherapy, radiation, hormonal medicines, immunotherapy, autoimmune illnesses, kidney issues, diabetes, or breastfeeding. Pathologists analyzed breast biopsies to confirm the precision of tumor staging and categorization. In order to assess the TNM staging procedure, breast cancer tumor staging was carried out in accordance with the American Joint Committee on Cancer (AJCC) criteria **(**15). Hormonal receptors HER2, ER and PR were among the prognostic/predictive biomarkers that were assessed using immunohistochemistry and, if required, silver in situ hybridization (SISH) utilizing paraffin blocks from breast core biopsy (16). The study was authorized by the Mansoura University Faculty of Medicine's Institutional Review Board in Egypt (code number: MS.23.11.2629). Following approval, all study participants were urged to submit signed informed consent.

Methods

Sample handling and collection

In a totally sterile process, five milliliters of peripheral venous blood were extracted from each study group using single-use plastic syringes. Each sample was separated into two tubes: 3 ml of blood was drawn without anticoagulant and spun for 15 minutes at 5000 rpm for biochemical and tumor marker testing, while 2 ml of blood was stored in a test tube containing ethylenediaminetetraacetic acid (EDTA) for hematological analysis.

Evaluation of lab parameters

Using colorimetric method kits and a bench colorimeter, the biochemical measurements, such as serum ALT, AST, albumin, bilirubin, alkaline phosphatase, creatinine, uric acid and lactate dehydrogenase were assessed. BioScien ARENA, Germany, produces lactate dehydrogenase. Additionally, ELISA kit was used to measure the serum BCL-2 concentration. Additionally, a complete hematological analyzer was used to evaluate the hematological measurements. Moreover, ELISA kits used to evaluate CEA and CA 15-3.

Statistical analysis

The data were analyzed using the SPSS. Parametric numerical data were presented by mean (± SD), non-parametric data were presented by median (range) and the non-numerical data was expressed by percentage and frequency. Student-t test was used to evaluate the statistical significance of the difference between study group parametric

variables. The Mann-Whitney U test was used to determine the statistical significance of a non-parametric variable of study groups. Chi-Square test was used to examine the relationship between qualitative variables as appropriate. ROC curve was applied for the diagnostic performance of Bcl2. P-value is considered significant if < 0.05.

3. Results and Discussion

Results

This study including 100 BC women with a mean age of 51.95 ± 11.84 years, and 100 cancer-free controls with a mean age of 53.78 ± 9.73 years. There was no statistically significant difference recorded in age between the BC patients and cancer-free controls (p = 0.23).

According to the hormonal receptors, the patients classified as, positive estrogen ER+ (76.0%), progesterone PR+ (67%), Her2+ (33%). Regarding the type of histopathology and grade of breast cancer (94.0%) of patients had Invasive Ductal Carcinoma (IDC), (6%) were invasive lobular carcinoma (ILC). Tumor grade II (83%). grade III (17%), as shown in **Table (1)**.

It was observed that 4% of patients had T0 stage, 22% had T1 stage, 54% had T2 stage, and 20% had T3 stage of breast tumor. It was noticed that 25% had N0 stage, 35% had N1 stage, 50% had N2 stage, and 25% had N3 stage of breast tumor. Seventy-two BC women had M0 stage, and 28% cases had M1 stage of tumor stage, as shown in **Table (2)**.

Current results showed that breast cancer patients had statistically lowered platelets count in comparison to the healthy group and significantly higher uric acid and tumor marker (CEA and CA15.3), LDH, GPT, GOT and bilirubin (p<0.05), **Table (3).**

In addition, serum Bcl2 levels were significantly higher in the BC patients compared to cancer-free controls [38 (29 – 55) vs. 4.5 (2.85 – 5.75), respectively, p<0.001], **Table (3).**

Validity of BCl2 test for diagnosing of breast cancer using ROC curve showed at a cut off value of 8.9, the sensitivity was 100% and specificity was 100%, at an (AUC=0.998, p<0.001), **Figure 1.**

The associations of BCL2 level with lab results **Tables (4)** and tumor characteristics **Table (5)** showed no significant difference.

Discussion:

The Bcl-2 protein family regulates both cell death and proliferation. High levels of Bcl-2 have been informed in several tumors (17). Several studies have found elevated levels of Bcl-2 protein in the serum in a number of malignancies such as ovarian cancer and lung cancer (18).

In this study, we found a statistically significant increase in serum Bcl-2 levels in BC patients, than the controls (p<0.001). Our results are consistent with those of the study by**,**suggesting that serum Bcl-2 could be used as a serum marker for disease diagnosis, and also suggesting a potential role for Bcl-2 in breast cancer development (19).

Our result is consistent with who demonstrated that Bcl-2 expression was upregulated in breast cancer patients compared with healthy blood donors. Hypomethylation and Bcl-2 gene rearrangement are two potential processes that could account for this increase. In breast cancer, estrogen has been shown to directly increase the Bcl-2 protein through transcriptional activation. Furthermore, in cancers of various kinds, there was an inverse relationship between Bcl-2 and the expression of Ki-**67 (20).**

Our results agreed with study that found serum BCL2 level was increased in the serum of Egyptian BC Women. Similarly, observed the elevation of serum BCL2 levels in breast cancer women before surgery compared with normal controls. Increased BCL2 levels in cancer cells suggest that this anti-apoptotic protein may play a crucial role in breast cancer progression. Overexpression of BCL2 protein in breast tumour cells may promote cell survival, resulting in tumour progression and metastases (21, 22).

Similary, prior to surgery, breast cancer patients had higher levels of Bcl-2 protein than normal controls, according to studies by and The elevated Bcl-2 levels in breast cancer cells suggest that this anti-apoptotic protein may play a crucial part in the development of breast cancer (19, 23).

According to**.** and**.** overexpression of the Bcl-2 protein may impact the survival of beneficial cells in breast tumor cells, ultimately resulting in tumor progression and metastasis. It was also discovered that downregulation of Bcl-2 in human breast cancer cells was linked to a favorable prognosis (24, 25).

Interestingly, also discovered that Bcl-2 positive was linked to shorter survival in the triple-negative (TNBC) group and longer survival in the non-TNBC group (26). Additionally, it was demonstrated by that Bcl-2 expression was present in both TNBC and non-TNBC patients, and that analyzing Bcl-2 expression could help choose therapeutic approaches for TNBC. The researchers observed that there is no known cure for this severe disease, although therapy approaches that target the Bcl-2 protein seem promising (27).

According to the most often utilized blood marker for breast cancer is CA15-3. Its primary applications at the moment are tracking newly diagnosed patients and tracking the course of treatment for individuals with more advanced disease. According to the results of the earlier investigation, individuals with breast cancer had noticeably greater levels of CA 15-3 than did healthy controls (28). In the meantime, tumor size and clinical stage were substantially correlated with serum CA 15-3. According to these findings, increased CA 15-3 levels seemed to be linked to tumor load or suggestive of malignant illness (29).

Our findings confirmed that the serum levels of Bcl-2 and CA 15-3 Abs were considerably greater in breast cancer patients than in controls. According to treatment resistance in a variety of cancer cells, including cases of breast cancer, was linked to elevated Bcl-2 expression levels (30).

About 75% of primary BC, with a preponderance of estrogen receptor-positive tumors, expresses high Bcl-2 levels (5). Bcl-2 is overexpressed in roughly 85% of estrogen receptor-positive tumors, 50% of HER2 receptor-positive tumors, 41% of triple-negative breast cancers, and 19% of basal-like tumors. These findings seem in line with gene expression profiling research, which indicates that tumors that express estrogen receptors are the main source of Bcl-2 expression. Interestingly, BRCA1-associated cancers had a lower rate of Bcl-2-positive tumors (31%) than cancers without BRCA1 mutations. This could be because BRCA1-associated cancers are triple-negative.

Earlier research found that Bcl-2 expression was linked to a better prognosis in breast cancer despite its anti-apoptotic qualities (31, 32). The expression of the Bcl-2 protein has a higher predictive value in hormone receptor-positive breast cancer than in hormone receptor-negative breast cancer, according to a substantial body of research (33). Nonetheless, some research has demonstrated that Bcl-2 expression is a separate predictor of outcome, even in triple-negative or hormone receptor-negative breast cancer.

Naoko et al. showed the benefit of Bcl-2 inhibitors in basal-like breast cancers expressing Bcl-2. This suggests that Bcl-2-targeted therapy may improve the poor clinical outcomes of patients with such Bcl-2-expressing tumors. This data backs up a clinical investigation of Bcl-2-targeted treatment for breast cancer. It is anticipated that Bcl-2 screening will enhance the ability to forecast clinical outcomes or the response to Bcl-2-targeted treatment in cases of breast cancer (34).

In conclusion, we concluded that serum Bcl2 and CA 15-3 levels are good biomarkers correlated with breast cancer.

Table (1): The tumor characteristics of BC cases.

Tumor characteristics		**BC (n=100)**	
		n	**%**
molecular classification	**ER+**	76.0	76.0%
	PR+	67.0	67%
	Her2+	33.0	33%
Tumor size	**small </=2cm**	21.0	21%
	large >2cm	79.0	79%
pathological type	**IDC**	94.0	94%
	ILC	6.0	6%
Tumor grade	**II**	83.0	83%
	III	17.0	17%

Parameters were expressed as frequency (percentage).

Table (2): The TNM stages of the BC cases

			BC n=100	
			n	%
Tumor staging	T	0	4.0	4%
		1	22.0	22%
		2	54.0	54%
		3	20.0	20%
	N	0	25.0	25%
		1	35.0	35%
		2	15.0	15%
		3	25.0	25%
	M	0	72.0	72%
		1	28.0	28%

Table (3): Comparison of laboratory parameters between the studied groups.

Parameter	*BC (n=100)*	*Control (n=100)*	*p-value*
TLC ($\times 10^9$/L)	7.32 ± 4.95	6.58 ± 4.47	*p=0.27*
Platelets ($\times 10^9$/L)	220 (16 – 450)	308.5 (200 – 483)	***p<0.001****
RBCs ($\times 10^{12}$/L)	4.09 ± 0.75	4.23 ± 0.58	*p=0.14*
Hemoglobin (g/dl)	11.54 ± 1.75	11.55 ± 1.74	*p=0.97*
GPT (U/L)	22.5 (5.8 – 147)	41 (33 – 61)	***p<0.001****
GOT (U/L)	30 (11 – 368)	23 (14 – 114)	***p<0.001****
Bilirubin (mg/dl)	0.5 (0.2 – 9)	0.4 (0.2 – 2.5)	***p=0.01****
Albumin (g/dl)	4 (1.91 – 5.3)	4 (2.37 – 5.18)	*p=0.45*
LDH (U/L)	233 (107 – 2081)	180 (105 – 210)	***p<0.001****
Creatinine (mg/dl)	0.8 (0.4 – 3.7)	0.8 (0.6 – 1.1)	*p=0.2*
Uric acid (mg/dl)	5 (1.3 – 16.3)	4 (2.1 – 6.9)	***p=0.001****
CEA (ng/ml)	4.58 (0.69 – 270)	1.37 (0.19 – 3.1)	***p<0.001****
CA15.3 (U/ml)	28.37 (4.9 – 850)	4 (3 – 8)	***p<0.001****
BCl2	38 (9.8 – 77)	4.5 (1 – 8)	***p<0.001****

*: Statistically significant (if *p<0.05*).

Table (4): Correlations of BCL2 level with lab results among BC group.

Parameter	BCL2	
	rs	*p*
GPT	-0.09	0.32
GOT	-0.11	0.27
bilirubin	-0.58	0.56
albumin	0.2	**0.03***
Creatinine	0.06	0.53
UA	0.07	0.48
CEA	0.1	0.3
CA15.3	0.01	0.9
LDH	0.05	0.61
WBCs	0.03	0.74
RBCs	0.02	0.78
Hemoglobin	0.04	0.69
Platelets	0.05	0.6
KI67	0.05	0.62

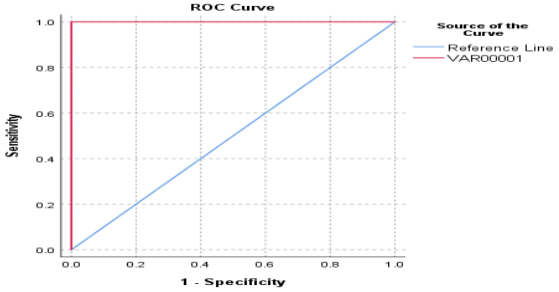

Figure 1: ROC curves of BCL2 for the diagnosis of BC

Table (5): Association of BCL2 with tumor characteristics among BC patients

Variable		BCL2	p-value
ER	**Positive**	38 (27.5 – 55.5)	p=0.48
	Negative	37.5 (30 – 54.5)	
PR	**Positive**	41 (29.1 – 56)	p=0.39
	Negative	35 (29 – 54.5)	
Her2	**Positive**	39 (29.5 – 49)	p=0.81
	Negative	38 (28.7 – 59)	
Tumor size	***small (<2cm)***	35 (29.9 – 50)	p=0.80
	large (>2cm)	39 (29 – 55)	
Tumor grade	***Early (T1-T2)***	36 (30.9 – 45)	p=0.90
	Late (T3-T4)	35 (33 – 46)	
Metastasis	***Absent***	40 (30.9 – 41.2)	p=0.77
	Present	34.5 (33 – 51)	
Histological grade	***Low (G1-G2)***	33.1 (31 – 42.5)	p=0.79
	High (G3)	34.5 (33 – 51)	
Pathological type	***IDC***	35.9 (31.65 – 40.2)	p=0.75
	ILC	32.1 (35.2 – 48)	

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