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The Diagnostic, Prognostic and Follow-up Value of Serum Bcl-2, Bax and p53 Proteins in Breast Cancer Patients: A Comparison with Serum CA 15-3

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Abstract

Background: Biomarkers accepted for clinical use in breast cancer have low sensitivity and specificity. Thus, there is a need for new markers to assist in the diagnosis, prognosis and follow-up of breast cancer patients. This study aims to investigate the diagnostic, prognostic and follow-up role of serum Bcl-2, Bax and p53 proteins in breast cancer patients in comparison with those of serum CA 15-3 as the most commonly used breast cancer marker.

Methods: We analyzed 50 breast cancer patients (before surgery, after one month of surgery and after six cycles of chemotherapy) and 50 normal healthy controls for serum Bcl-2, Bax, p53 and CA 15-3 levels.

Results: Mean serum Bcl-2 and CA 15-3 levels significantly increased, whereas the mean serum p53 level significantly declined in breast cancer patients compared to normal healthy controls. Using the ROC curve analysis, serum p53 had the greatest area under the curve (85.6%). Serum Bcl-2 levels significantly decreased after six cycles of chemotherapy compared with its level one month after surgery. Preoperative serum levels of Bcl-2, Bax, p53 and CA 15-3 were non-significantly correlated with patient's disease-free survival.

Conclusion: Serum p53 was superior to Bcl-2 and CA 15-3 in the diagnosis of breast cancer patients. Only Bcl-2 could be used for monitoring the effect of chemotherapy on breast cancer patients. None of the assayed biomarkers had a role in monitoring the effect of surgery on breast cancer patients. None of the assayed biomarkers had a prognostic role for breast cancer patients.

Keywords: Breast cancer, Apoptosis, Bcl-2, Bax, p53, CA 15-3, Diagnosis, Prognosis, Follow-up

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Introduction

In Egypt, breast cancer is the most common female malignancy. In Alexandria, it accounts for 42.7% of all malignancies in females.[1] Apoptosis occurs in several normal and pathological processes. The mechanisms of apoptosis are highly complex involving an energy-dependent cascade of molecular events. There are two main apoptotic pathways: extrinsic (death receptor pathway) and intrinsic (mitochondrial pathway).[2-4]

The Bcl-2 family of proteins regulates apoptosis, cell cycle and differentiation. This family is divided into two groups, anti-apoptotic (e.g., Bcl-2 protein) and pro-apoptotic (e.g., Bax) which work together to control healthy cells.[5] Bcl-2 protein can inhibit the mitochondrial apoptotic pathway via binding to its pro-apoptotic protein Bax, thereby preventing the release of cytochrome c and activation of the apoptotic cascade.[6]

Bax promotes apoptosis, induces permeabilization of the outer mitochondrial membrane (OMM), controls transition of cytochrome c through the OMM and maintains endoplasmic reticulum Ca^{2+} levels.[7]

For apoptosis to occur, the Bax protein concentration should be greater than the Bcl-2 protein. Studies have shown reductions in Bax to be associated with a poor response to chemotherapy in metastatic breast cancer.[8] Bax expression is induced by γ-radiation, chemotherapeutic drugs and the p53 protein.[9,10]

p53 is a poly-functional tumor suppressor protein essential for preventing inappropriate cell proliferation and maintaining genome integrity following genotoxic stress. Following various intracellular and extracellular stimuli, such as DNA damage, heat shock, hypoxia, and oncogene overexpression, p53 is activated and induces either cell cycle arrest in cases of repairable damage or apoptosis in cases of severe damage. p53 induces apoptosis by stimulation of Bax activation and down-regulation of Bcl-2.[11-13]

Cancer antigen 15-3 (CA 15-3) is a circulating breast cancer-associated antigen.[14] Preoperative measurement of CA 15-3 is of little value in the early detection of breast cancer and thus can not be used for its early diagnosis.[15] However it can be used for monitoring the response of breast cancer patients to therapy and in detecting recurrent disease.[14]

The aim of this work was to investigate the diagnostic, prognostic and follow up roles of serum Bcl-2, Bax and P53 proteins in breast cancer patients in comparison with those of serum CA 15-3 as the most commonly used breast cancer marker.

Patients and Methods

One hundred premenopausal females in this case-control study were enrolled. Females were divided into two groups. Group I included 50 female patients with clinical stages II and III invasive ductal carcinoma of the breast[16] that was recently detected. Patients had not undergone surgery, nor did they receive chemotherapy. Their mean age was 41.73±12.2 years. Patients were recruited from the Departments of Experimental and Clinical Surgery and Cancer Management and Research of the Medical Research Institute, Alexandria University in the period from January 2010 to August 2010. Group II (normal healthy control group) included 50 normal healthy female volunteers of comparable age (40.18±11.05 years), menstrual cycle and socioeconomic status as the patients.

After approval from the Ethical Committee, Medical Research Institute, Alexandria University, Egypt, signed informed consents were obtained from all subjects who agreed to participate in the study. Each patient underwent full history recording, thorough clinical examination, routine laboratory investigations that included complete blood count (CBC), radiological investigations that included breast mammography, ultrasonography of the abdomen and liver, chest X-ray, CT scan and bone scan when needed, in addition to a fine needle aspiration cytology (FNAC) of the breast mass to establish the pathological diagnosis.

Clinicopathologic data obtained from patients' pathology reports included tumor

Table 1. ROC curve-based characteristics for serum Bcl-2, p53 and CA 15-3 in breast cancer patients before surgery.

Variables	AUC** (%)	*P*-value	Cut-off value	Sensitivity (%)	Specificity (%)
p53 (U/ml)	85.6	<0.05*	2.82	80	87
Bcl-2 (ng/ml)	84.1	<0.05*	1.47	77	76
CA 15-3 (IU/ml)	76.1	<0.05*	25	47	100

*Significance was considered as $P<0.05$; **AUC: Area under the curve

pathological grade, axillary lymph node involvement, vascular invasion, estrogen receptor status (ER), progesterone receptor status (PR) and Her-2 expression. Each breast cancer patient's clinical stage was determined by the oncologist according to the tumor-node-metastasis (TNM) classification system.[16]

All 50 breast cancer patients underwent modified radical mastectomies,[17] followed by adjuvant combination chemotherapy [5-fluorouracil, adriamycin and cyclophosphamide (FAC)][18] for six cycles. After six cycles of chemotherapy, breast cancer patients were evaluated clinically, laboratory and radiologically to estimate the clinical response. The patients were followed up clinically for two years for observation of disease-free survival (DFS; local recurrence or metastasis).

Laboratory investigations

Blood samples were collected once from normal healthy female volunteers and thrice from breast cancer patients at the times before surgery, after one month of surgery and after six cycles of chemotherapy. Immediately after withdrawing blood from the patients, the samples were allowed to coagulate, then centrifuged for 20 min at 3500 rpm. The separated sera were aliquoted, frozen at -80°C, and stored until assayed. After thawing, each serum aliquot was assayed only once. Determination of serum levels of Bcl-2, Bax, p53 proteins and CA 15-3 were carried out at the Radioisotopes Laboratory of the Radiation Sciences Department, Medical Research Institute, Alexandria University, Egypt.

Determination of serum Bcl-2 levels

Human serum Bcl-2 levels were quantified using a ready-for-use ELISA kit (eBioscience, UK) according to the manufacturer's protocol. Briefly, diluted sera were added to the corresponding sample microwells, followed by the addition of a diluted biotin conjugate solution. Samples were allowed to incubate for 2 h at room temperature. After washing, a diluted streptavidin-HRP solution was added and the samples were incubated at room temperature for 1 h. After washing, TMB substrate solution was added and the samples were incubated at room temperature for 10 min in the dark. The reaction was stopped by the addition of 1 M phosphoric acid. Absorbance was measured at 450 nm. We determined Bcl-2 serum levels by using a standard curve. The sensitivity of the assay was 0.33 U/ml.

Determination of serum Bax levels

Serum Bax levels were determined using a ready-for-use ELISA kit (USCN, USA) according to the manufacturer's protocol. Briefly, sera were added to the corresponding sample microwells, followed by incubation for 2 h at 37°C. After decantation, the detection reagent A was added to each well followed by incubation for 1 hr at 37°C. After decantation and washing, detection reagent B was added to each well followed by incubation for 30 minutes at 37°C. After decantation and washing, substrate solution was added to each well followed by incubation for 15-25 minutes at 37°C in the dark. The reaction was terminated by addition of stop solution and absorbance was measured at 450 nm using an ELISA reader. Serum Bax levels were determined using a standard curve.

Determination of serum p53 levels

Human serum p53 levels were determined using a ready-for-use ELISA kit (eBioscience, UK) according to the manufacturer's protocol.

Briefly, diluted sera were added to the corresponding sample microwells, followed by the addition of a diluted biotin conjugate solution and incubation for 2 h at room temperature. After washing, a diluted streptavidin-HRP solution was added, followed by incubation at room temperature for 1 h. After washing, a TMB substrate solution was added and the samples were allowed to incubate at room temperature for 10 min in the dark after which the reaction was stopped by the addition of 1 M phosphoric acid. Absorbance was measured at 450 nm. The p53 serum concentration was determined by referring to a standard curve. The sensitivity of the assay was 0.33 U/ml.

Determination of serum CA 15-3

The level of serum CA 15-3 was determined using a ready-for-use Immunoradiometric Assay (IRMA) kit (Diasource, Belgium) according to the manufacturer's protocol. Briefly, serum was added to a plastic tube coated with the capture antibody Mab1 and agitated for 90 min at room temperature. After washing, 125 Iodine-labeled anti-CA 15-3 antibody (Mab2) was added and the reaction tubes were agitated for 90 min at room temperature. After washing, the bound radioactivity in each tube was counted in a gamma counter (Perkin Elmer, Finland) for 60 sec. Computer assisted data reduction was used to simplify the calculations. The 5-parameter logistic function curve was used to calculate CA 15-3 levels in each serum sample.

Statistical analysis

Statistical analysis was performed using the SPSS 11.5 software package. We used the non-parametric Mann–Whitney U-test to study differences between breast cancer patients and controls regarding serum Bcl-2, Bax, p53 and CA 15-3 levels. The non-parametric Kruskal-Wallis test was used to study the differences in serum parameters before and after surgery, and after chemotherapy. The non-parametric Spearman's test was used to investigate correlations between different serum parameters. The diagnostic values of serum Bcl-2, P53 and CA 15-3 were compared using the Receiver Operating Characteristic (ROC) curve analysis. Univariate survival analysis of the studied parameters was assessed using the Kaplan Meier method and the log rank test was used for survival time. Statistical differences between survival curves were evaluated by the log-rank test. We considered *P*-values less than 0.05 to be significant.

Results

Bcl-2 (ng/ml) serum levels in normal healthy controls and breast cancer patients before surgery, after one month of surgery and after six cycles of chemotherapy

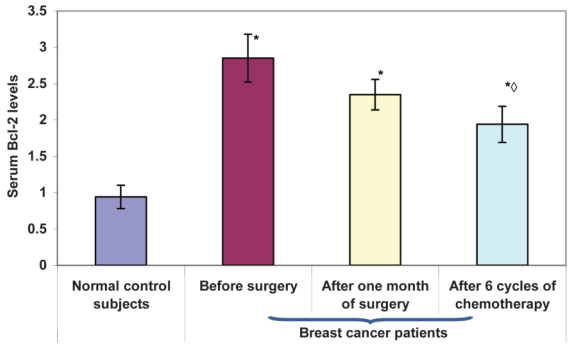

Figure 1: Mean±S.E. Bcl-2 levels (ng/ml) in normal control subjects and breast cancer patients before surgery, after one month of surgery and after six cycles of chemotherapy; *Significantly different from the control group; ◊Significantly different from the breast cancer patient group after one month of surgery. Significance was considered at the level of $P<0.05$.

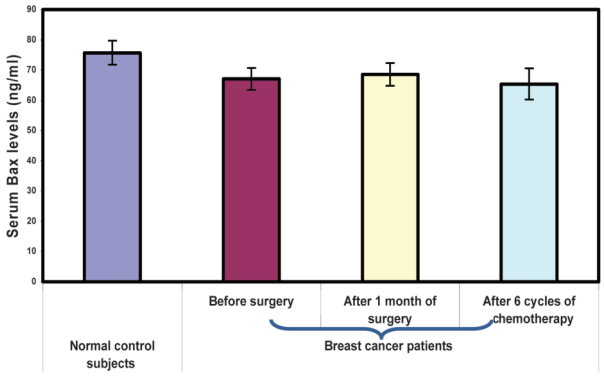

Figure 2. Mean±SE Bax (ng/ml) levels in normal control subjects and breast cancer patients before surgery, after one month of surgery and after six cycles of chemotherapy.

Table 2. Correlations between serum p53, Bcl-2 and Bax levels of breast cancer patients before surgery, after one month of surgery and after six cycles of chemotherapy.

			Bcl-2 (ng/ml)	Bax (ng/ml)
p53 (U/ml)	Before surgery	r	-0.415	0.116
		p	0.023*	0.381
	After 1 month of surgery	r	0.096	0.125
		p	0.614	0.512
	After 6 cycles of chemotherapy	r	0.089	0.069
		p	0.642	0.717

*Significance was considered to be $P<0.05$

The mean±SE levels of serum Bcl-2 were 0.94±0.16 ng/ml in normal control subjects, 2.85±0.33 ng/ml in breast cancer patients before surgery, 2.35±0.21 ng/ml one month after surgery and 1.94±0.25 ng/ml after six cycles of chemotherapy. Serum Bcl-2 levels in breast cancer patients before surgery, one month after surgery and after six cycles of chemotherapy were significantly higher than those of the control group ($P<0.05$). Surgical removal of the breast had a non-significant effect on serum Bcl-2 levels ($P>0.05$). Six cycles of chemotherapy resulted in a significant reduction of serum Bcl-2 levels compared to one month after surgery ($P<0.05$; Figure 1).

The mean±SE serum Bax levels were 75.7±3.94 ng/ml in normal control subjects, 67.1±3.6 ng/ml in breast cancer patients before surgery, 68.5±3.74 ng/ml one month after surgery and 65.37±5.21 ng/ml after six cycles of chemotherapy. These results revealed that the level of serum Bax in breast cancer patients either before or after one month of surgery as well as after six cycles of chemotherapy were approximately within the same range and non-significantly less than those of the control group ($P>0.05$). Surgical removal of the breast and six cycles of chemotherapy had no significant effects on serum Bax levels ($P>0.05$; Figure 2).

Serum Bax (ng/ml) levels in normal healthy controls and breast cancer patients before surgery, after one month of surgery and after six cycles of chemotherapy

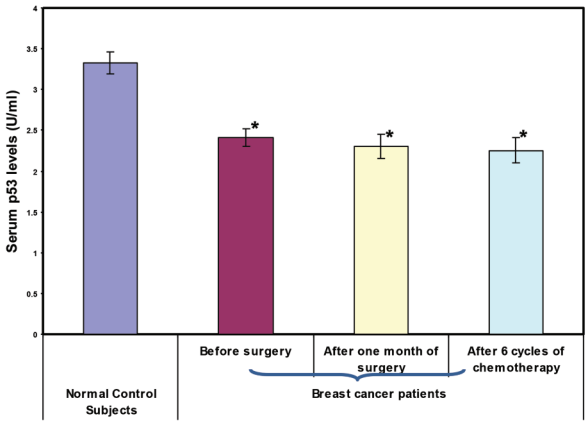

Figure 3. Mean±SE p53 (U/ml) levels in normal control subjects and breast cancer patients before surgery, after one month of surgery and after six cycles of chemotherapy; *Significantly different from the control group; Significance was considered at the level of $P<0.05$.

Serum levels of p53 (U/ml) in normal healthy controls and breast cancer patients before surgery, after one month of surgery and after six cycles of chemotherapy

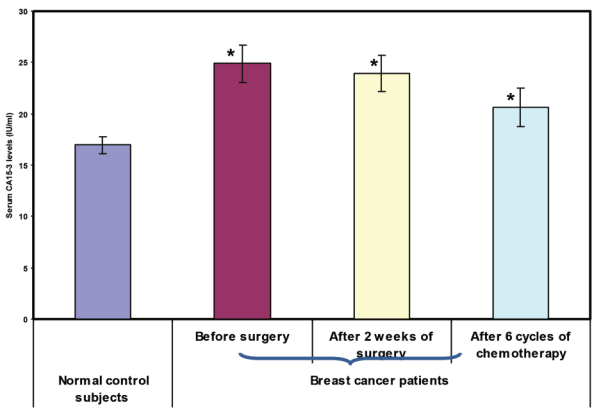

Figure 4. Mean±SE CA 15-3 (IU/ml) levels in normal control subjects and breast cancer patients before surgery, after one month of surgery and after six cycles of chemotherapy; *Significantly different from the control group; Significance was considered at the level of $P<0.05$.

The mean±SE serum p53 levels were 3.33±0.13 U/ml in normal control subjects, 2.41±0.10 U/ml in breast cancer patients before surgery, 2.31±0.14 U/ml one month after surgery and 2.26±0.15 U/ml after six cycles of chemotherapy. These results revealed that the levels of serum p53 of breast cancer patients before surgery, after one month of surgery as well as after six cycles of chemotherapy were significantly less than those of the control group (P<0.05). Surgical removal of the breast and six cycles of chemotherapy had non-significant effects on serum p53 levels (P>0.05; Figure 3).

Serum CA 15-3 (IU/ml) levels in normal healthy controls and breast cancer patients before surgery, after one month of surgery and after six cycles of chemotherapy

The mean±SE serum CA 15-3 levels were 16.94±0.84 IU/ml in normal control subjects, 24.92±1.82 IU/ml in breast cancer patients before surgery, 23.95±1.77 IU/ml one month after surgery and 20.61±1.89 IU/ml after six cycles of chemotherapy. These results revealed that serum CA 15-3 levels in breast cancer patients either before or after one month of surgery were approximately within the same range and significantly higher than those of the control group. Surgical removal of the breast had no significant effect on serum CA 15-3 levels (P>0.05). After six cycles of chemotherapy, the CA 15-3 level non-significantly decreased compared with its level after one month of surgery, however it was still significantly higher than its level in the control group (Figure 4).

Comparison of the diagnostic values of Bcl-2, p53 and CA 15-3 levels in breast cancer patients before surgery using the receiver operating characteristic (ROC) curve analysis

Receiver operating characteristic curve analysis was used to compare the diagnostic values of Bcl-2, p53 and CA 15-3 depending on the area under the ROC curve (AUC). The higher AUC corresponds to a better diagnostic test. Serum Bcl-2 showed a significant AUC (84.1%, P<0.05) with a 77% sensitivity and 76% specificity at a cut-off value of 1.47 ng/ml. Serum p53 showed a significant AUC (85.6%, P<0.05) with 80% sensitivity and 87% specificity at a cut-off value of 2.82 U/ml. Serum CA 15-3 showed a significant AUC (76.1%, P<0.05) with 47% sensitivity and 100% specificity at a cut-off value of 25 IU/ml (Table 1; Figures 5 and 6).

Correlations between serum Bcl-2, Bax and p53 levels with breast cancer clinicopathological features before surgery

Serum Bcl-2 showed significant indirect correlations with ER status (r= -0.438, P=0.015) and Her-2 expression (r= -0.369, P=0.045). Serum p53 showed a significant direct correlation with ER status (r= 0.395, P=0.031) and a significant indirect correlation with vascular invasion (r=-0.513, P=0.004). Serum CA 15-3 showed significant direct correlations with tumor size (r=0.464, P=0.01) and

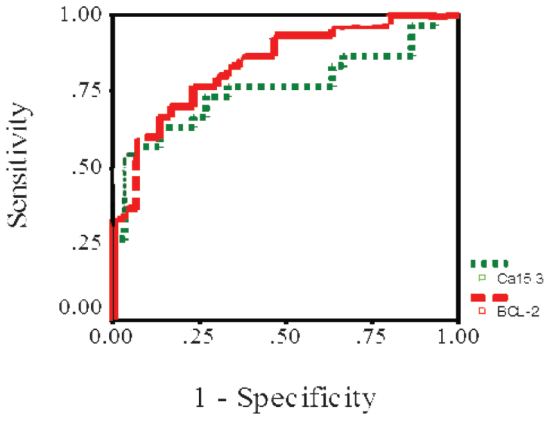

Figure 5. ROC curves for serum Bcl-2 and CA 15-3 in breast cancer patients before surgery.

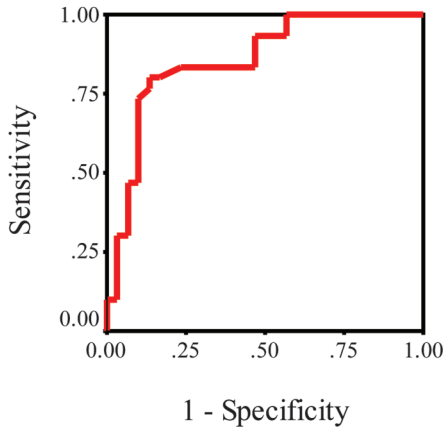

Figure 6. ROC curve for serum P53 in breast cancer patients before surgery.

clinical stage (r=0.408, *P*=0.028).

Correlations between serum Bcl-2, Bax and p53 levels in breast cancer patients before surgery, after one month of surgery and after six cycles of chemotherapy

According to Table 2, serum p53 showed a significant indirect correlation with serum Bcl-2 before surgery. The other correlations were non-significant.

The prognostic value of serum Bcl-2, p53 and CA 15-3 in breast cancer patients before surgery

To study the prognostic value of these parameters, we constructed Kaplan-Meier DFS curves. After the completion of six chemotherapy cycles, patients were followed clinically, radiologically and via laboratory analyses for 24 months for observation of any local recurrence or metastases. Four patients had metastases, whereas 46 patients were free of metastases. As shown in Figures 7-9, Kaplan-Meier survival curves for breast cancer patients before surgery revealed that patients with elevated serum Bcl-2 and CA 15-3 levels had DFS that was non-significantly shorter than patients with low levels according to log-rank *P*-values (*P*=0.34 and *P*=0.069, respectively). Patients with lower levels of serum p53 had DFS that was non-significantly shorter than those with higher levels according to the log-rank *P*-value (*P*=0.5).

Discussion

Various biomarkers are used in conjunction with clinicopathological features to identify patients at higher risk of relapse.[19] Biomarkers accepted for clinical use in breast cancer, such as CA 15-3, CEA and CA 27-29, have low sensitivity and specificity, and are thus more useful for patients at advanced stages of breast cancer rather than for early cancer diagnosis. Thus, there is a need for new markers to assist with diagnosis and prognosis of primary breast cancer.[20]

Over the last several years, research on the role of apoptosis in malignancy in general and in breast cancer in particular has increased.[21,22] Apoptotic markers are now being investigated and have a role in detecting the progression of cancer and its response to various chemotherapeutic agents.[23]

The apoptotic process is controlled by several genes. The balance between expressions of these genes regulates the cell cycle and apoptosis. This balance is regulated by other stimuli such as p53 protein or estrogen receptors in breast carcinomas. Excess Bcl-2 promotes cell survival by inhibiting apoptosis, whereas excess Bax accelerates cell death.[24] Therefore, in the present study, serum Bcl-2, Bax, p53 and CA 15-3 have been quantified and their predictive, prognostic and follow-up roles were compared in breast cancer patients.

Bcl-2 is a protein that has the capability to block most pro-apoptotic stimuli,[25] and therefore promotes cell survival. Bcl-2 regulates the intrinsic mitochondrial apoptotic pathway that responds to numerous stress stimuli such as DNA damage or deprivation of growth factors. It maintains the

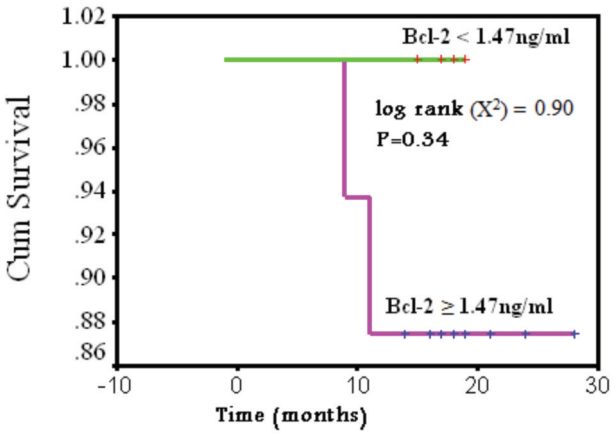

Figure 7. Kaplan-Meir DFS curve of breast cancer patients in relation to serum Bcl-2 before surgery.

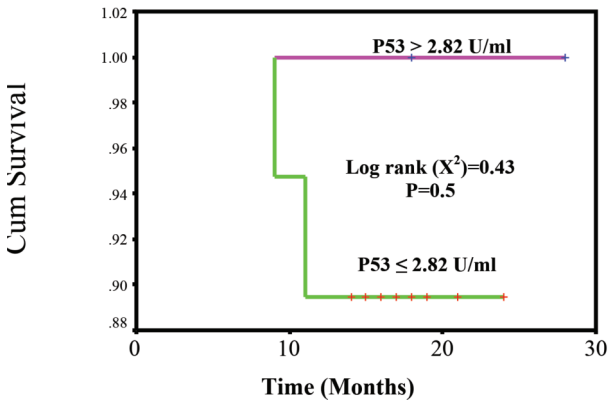

Figure 8. Kaplan-Meir DFS curve of breast cancer patients in relation to serum P53 before surgery.

integrity of mitochondria by preventing pro-apoptotic proteins such as Bax or Bak from initiating this pathway.[26]

In the present study, there were significantly higher serum Bcl-2 levels in breast cancer patients before surgery than in normal healthy controls. According to this result, serum Bcl-2 could be used to differentiate breast cancer patients from normal healthy controls. Our results agreed with those of Mahdy et al.[20] and Kallel-Bayoudh et al.[27] who reported high levels of Bcl-2 in breast cancer patients before surgery compared with normal controls.

The increase of Bcl-2 in cancer cells points to a potentially critical role of this anti-apoptotic protein in breast cancer progression. Overexpression of Bcl-2 protein may serve as a determinator of an advantageous cell survival in breast tumor cells, ultimately leading to tumor progression and metastases. This hypothesis has previously been supported by in vivo experimental studies.[28, 29]

Previous studies reported the expression of human Bcl-2 in 80% of breast cancers and showed significant direct correlation with the expression of ER and PR, good prognostic features in breast cancer.[30] This surprising association between the apoptosis inhibitor and good prognostic features was confirmed by improved survival of patients with tumors that were Bcl-2 positive compared with Bcl-2 negative tumors. However, in the current study, serum Bcl-2 showed a significant indirect correlation with ER expression. Our finding could be confirmed from in vitro and in vivo studies where Bcl-2 contributed to cell transformation and tumor progression, as indicators of a bad prognosis in breast cancer.[31] It has been suggested that Bcl-2 protein overexpression may have a pro-apoptotic function in some circumstances by increasing the half-life of the Bax protein.[32]

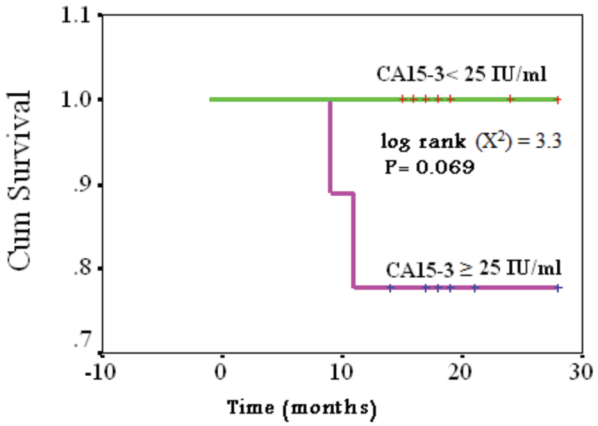

Figure 9. Kaplan-Meir DFS curve of breast cancer patients in relation to serum CA15.3 before surgery.

At one month following a modified radical mastectomy, we expected that the level of Bcl-2 would be decreased compared to its pre-surgery level, however the comparative analysis showed a non-significant difference. In view of these results, two possible probabilities may be responsible for maintaining a high level of Bcl-2. The first may be the presence of free circulating malignant cells that have already escaped from the primary tumor. The second may be due to incomplete clearance of this protein from the circulation.

The results of the current study demonstrated that the level of serum Bcl-2 after six cycles of chemotherapy significantly decreased compared to its level one month after surgery. It is well known that chemotherapeutic drugs kill cancer cells by inducing apoptosis. As Bcl-2 is an anti-apoptotic protein, its decline after six cycles of chemotherapy means that the interaction with Bcl-2 may play a prominent role in the mechanism of action of chemotherapeutic drugs.[33] These results may lead us to predict that some chemotherapeutic regimens, including FAC, induce apoptosis by decreasing Bcl-2 levels.

After completion of six chemotherapy cycles, patients were followed clinically, with radiological and laboratory analyses, for 24 months for the presence of recurrence or metastases. Four patients had metastases, whereas 46 patients were free from metastases. Thus. the FAC combination chemotherapy used in this study might be effective in killing and removing residual tumor cells that remain after mastectomy, in addition to the prevention of metastases and local recurrence. According to these results, protection from apoptotic cell death by Bcl-2 could prevent these drugs from working properly; perhaps the

circulating breast cancer cells would be the source of metastases.

In the present study we found a non-significant correlation between preoperative serum Bcl-2 protein levels and DFS of the patients. These results confirmed those of Gaballaah et al.[34]

Bax is one of the main effectors in breast cancer. However, in contrast with the Bcl-2 protein, there are relatively few clinical studies on the biological role of Bax in breast cancer. The balance between the expressions of both genes is an important regulator of apoptosis.[35] In the present study, we have determined that serum Bax levels in breast cancer patients either before or after one month of surgery as well as after six cycles of chemotherapy approximated those of the control group. These results supported the histopathological findings of Vargas-Roig et al.[36] who stated that intensity of nuclear Bax expression was weak or moderate in breast cancer cells, with no significant change after drug administration. In addition we found non-significant correlations between serum Bax levels and the clinicopathological features of breast cancer patients.

The tumor suppressor p53 protein is an important negative regulator of cellular proliferation. The p53 gene product is induced in response to DNA damage. Evidence has shown that the expression of p53 protein leads to cell cycle arrest in G1 to allow for DNA repair and, in some cases, to apoptosis to prevent the replication of damaged DNA.[37,38] Alterations in p53 lead to loss of its cell growth regulatory function, resulting in accelerated cell growth and increased rate of DNA mutation. The unchecked propagation of these mutations is thought to contribute to the development of human cancers.[39]

The results of the present study showed significantly lower serum p53 levels in breast cancer patients than normal healthy controls. Our results contradicted those of Balogh et al.[40] who found that the positive rates of p53 protein in breast cancer patients were significantly higher than normal healthy controls. This contradiction might be due to the fact that p53 is a tumor suppressor protein with pro-apoptotic properties, so it is logical for p53 to be lower in breast cancer patients compared with normal healthy controls, which supports the findings of the present study.

The present study showed a significant direct correlation between p53 and the expression of ER. These results agreed with those by Nadasi et al.[41] However, no significant change was observed in serum p53 levels after one month of surgery and after six cycles of chemotherapy. Thus, the p53 protein has no role in monitoring the response of breast cancer patients to surgery and chemotherapy.

In the present study, serum p53 showed a significant indirect correlation with the vascular invasion ability of breast cancer cells. As p53 is a tumor suppressor protein, its decline in breast cancer cells may promote the progression and metastasis of these cells to other organs. Our results have confirmed those by other authors who suggested that p53 mutations can be used as a predictor of risk for breast cancer, since p53 mutations are more likely to be found in highly-invasive, poorly-differentiated, high-grade breast tumors.[42,43]

In the present study we found a non-significant correlation between preoperative serum p53 protein levels and DFS of the patients. These results confirmed those of Gunel et al.[44] who found a non-significant correlation between DFS and p53. In contrast to our results, Allred et al.[45] found that p53 was a significant predictor of reduced DFS.

Several studies reported a significant indirect correlation between Bcl-2 and p53 in breast cancer.[46, 47] The current study has confirmed this indirect correlation between Bcl-2 and p53 suggesting that p53 is a negative regulator of Bcl-2 expression and the mutation or in-activation of p53 is somehow related to regulation of Bcl-2 in breast cancer patients.

CA 15-3 is the most widely used serum marker in breast cancer.[48] Currently, its main uses are in surveillance of patients with diagnosed disease and monitoring the treatment of patients with advanced disease.[49] The results of the present study have shown significantly higher CA 15-3 levels in

breast cancer patients than normal healthy controls. At the same time, serum CA 15-3 significantly correlated with tumor size and clinical stage. These results indicated that high levels of CA 15-3 appeared to be related to tumor burden or implied the presence of malignant disease. These results supported the study of Hewala et al.[50]

One month following modified radical mastectomy, we expected that the level of CA 15-3 would be significantly decreased compared to its level before surgery. However a comparative analysis showed a non-significant decline. However, this decline was significantly higher than its level in normal healthy controls. This result might be attributed to the long half-life of CA 15-3 as previously reported by Kerin et al.[51] who stated that, since CA 15-3 has a long half life, a significant drop in CA 15-3 might not become obvious until three months after removal of the bulk mass of the tumor. From this clinical point of view, our results showed that serum CA 15-3 had no role in monitoring the response of breast cancer patients to surgery.

Also, a non-significant decline was observed in serum CA 15-3 levels after six cycles of chemotherapy compared with its level one month after surgery, which was significantly higher than its level in normal healthy controls. Although many studies[52] have reported that serum CA 15-3 plays a role in monitoring the response of breast cancer patients to chemotherapy, the absence of this role of CA 15-3 in the present study may be due to: 1) the small sample size and 2) the toxic effect of the chemotherapy on normal epithelia, which manifests clinically as hand-foot syndrome.[53]

In the present study we found a non-significant correlation between preoperative serum CA 15-3 levels and DFS of the patients. Although our results contradicted many studies,[54] this contradiction might be due to the small sample size and the short follow-up duration (24 months) of the current study.

In the present study, serum Bcl-2, p53 and CA 15-3 were significant predictive markers for breast cancer patients. To compare their predictive value, ROC curve analysis was applied in such a way that the higher AUC corresponded to the superior diagnostic marker. Serum p53 showed the greatest AUC (85.6%) followed by Bcl-2 (84.1%) and CA 15-3 (76.1%). Regarding the AUC for Bcl-2, our results confirmed those by Mahdy et al.[20] who found a nearly typical AUC of 84.2% for Bcl-2.

For p53, at a cut-off value 2.82 U/ml, there was a sensitivity of 80% and a specificity of 87%. Bcl-2, at a cut-off value 1.47 ng/ml, had 77% sensitivity and 76% specificity. For CA 15-3, at a cut-off value 25 IU/ml, the sensitivity was 47% and specificity was 100%. These results suggested that serum p53 was superior to serum Bcl-2 and CA 15-3 for prediction of breast cancer in patients. Although serum p53 and Bcl-2 proteins have been investigated in breast cancer patients, to the best of our knowledge, this is the first study that compares the diagnostic value of serum p53 and Bcl-2 proteins with that of serum CA 15-3, with a determination of the precise cut-off value, sensitivity and specificity of each protein in breast cancer patients.

Conclusion

From this study, we concluded that serum Bcl-2, p53 and CA 15-3 were good diagnostic biomarkers for breast cancer patients. However, of these, p53 was the best. Only serum Bcl-2 could be used for monitoring the effect of chemotherapy on breast cancer patients. None of the assayed biomarkers had a role in monitoring the effect of surgery and none have shown a preoperative prognostic role in breast cancer patients. Further studies with larger sample sizes are required to establish the diagnostic, follow-up and prognostic role of the assayed parameters in breast cancer patients and in other tumors.

References

1. Alexandria Cancer Registry Annual Report. Medical Research Institute, Alexandria University, Egypt, 2010.
2. Huang WP, Klionsky DJ. Autophagy in yeast: A review of the molecular machinery. *Cell Struct Funct* 2002;27(6):409-20.
3. Martinvalet D, Zhu P, Lieberman J. Granzyme A induces caspase independent mitochondrial damage, a required first step for apoptosis. *Immunity*

2005;22(3):355-70.
4. Elmore S. Apoptosis: A review of programmed cell death. *Toxo Path* 2007;35(4):495-516.
5. Reed JC. Bcl-2–family proteins and hematologic malignancies: History and future prospects. *Blood* 2008; 111(7):3322-30.
6. Teijido O, Dejean L. Upregulation of Bcl-2 inhibits apoptosis driven Bax insertion but favors Bax relocalization in mitochondria. *FEBS Lett* 2010;584(15):3305-10.
7. Hoppins S, Edlich F, Cleland MM, Banerjee S, McCaffery JM, Youle RJ, et al. The soluble form of Bax regulates mitochondrial fusion via MFN2 homotypic complexes. *Mol Cell* 2011;41(2):150-60.
8. Kim R. The roles of Bcl-2 and Bcl-xl in cell death. *Biochem Biophys Res Commun* 2005;33(2):336-43.
9. Tones RG, Bui T, White C, Madesh M, Krawczyk CM, Lindsten T, et al. The proapoptotic factors Bax and Bak regulate T cell proliferation through control of endoplasmic reticulum Ca^{2+} homeostasis. *Immunity* 2007;27(2):268-80.
10. Wolter KG, Hsu Y, Smith CL, Mechushtan A, Xi X, Youle RJ. Movement of Bax from cytosol to mitochondria during apoptosis. *J Cell Biol* 1997;139(5):128-92.
11. Vousden KH, Lu X. Live or let die: The cell's response to p53. *Nat Rev Cancer* 2002;2(8):594-604.
12. Zhou J, Ahn J, Wilson SH, Prives C. A role for p53 in base excision repair. *EMBO J* 2001;20(4):914-23.
13. Hofseth LJ, Hussain SP, Harris CC. p53: 25 years after its discovery. *Trends Pharmacol Sci* 2004;25(4):177-81.
14. Keyhani M, Nasizadeh S, Dehghannejad A. Serum CA 15-3 measurement in breast cancer before and after mastectomy. *Arch Iranian Med* 2005;8:263-6.
15. Duffy MJ. Ca 15-3 and related mucins as circulating markers in breast cancer. *ANN Clin Biochem* 1999;36(Pt 5):579-86.
16. Haskell CM, Lowitz BB, Casciato AD. Breast cancer. In: Casciato AD and Lowitz BB, eds. Manual of clinical oncology, 2nd ed. Boston, Toronto (pub.): Little and Brown Company, 1985:150-65.
17. Rintoul RF. Operations on the breast. In: Farquhaerison's text book of operative surgery, 7th ed. New York: Churchill Livingstone, Inc. 1986:270-81.
18. Abeloff MD, Lichter AS, Niederhuber JE, Pierce LJ, Aziz DC. Breast. In: Abeloff MD, Armitage JO, Licher AS, Niederhuber JE, eds. Clinical oncology. NewYork: Churchill Livingstone, Inc. 1995:Chapter 73, pp 1617-714.
19. Purdom M, Cibull ML, Stratton TD, Samayoa LM, Romond EH, Mcgrath PC, et al. Should histologic grade be incorporated into the TNM Classification System for small (T1, T2) node-negative breast adenocarcinomas? *Patholog Res Int* 2010; Oct 26;2011: 825627.
20. Mahdy EM, Shousha WG, Ahmed HH, Metwally FM, Ramadan SS. Significance of serum HGF, Bcl-2 and nitric oxide in primary breast cancer. *Nat Sci* 2011;9(5):34-41.
21. Munakata S, Watanabe O, Ohashi K, Morino H. Expression of Fas ligand and Bcl-2 in cervical carcinoma and their prognostic significance. *Am J Clin Pathol* 2005;123(6):879-85.
22. Zhang L, Levi E, Majumder P, Yu Y, Aboukameel A, Du J. Transactivator of transcription-tagged cell cycle and apoptosis regulatory protein-1 peptides suppresses the growth of human breast cancer cells *in vitro* and *in vivo*. *Mol Cancer Ther* 2007;6(5):1661-72.
23. Walker PR, Saas P, Dietrich PY. Role of Fas ligand (CD95L) in immune escape: The tumor cell strikes back. *J Immunol* 1997;158(10):4521-4.
24. Boise LH, Gottschalk AR, Quintans J, Thompson CB. Bcl-2 and Bcl-2 related proteins in apoptosis regulation. *Curr Top Microbiol Immunol* 1995;200:107-21.
25. Agarwal ML, Taylor WR, Chernov MV. The p53 network. *J Biol Chem* 1998;273(1):1-4.
26. Frenzel A, Grespi F, Chmelewskij W. Bcl-2 family proteins in carcinogenesis and the treatment of cancer. *Apoptosis* 2009;14(4):584-96.
27. Kallel-Bayoudh I, Hassen HB, Khabir A, Boujelbene N, Daoud J, Frikha M, et al. Bcl-2 expression and triple negative profile in breast carcinoma. *Med Oncol* 2011;28 Suppl 1:S55-61.
28. Kumar R, Vadlamudi RK, Adam L. Apoptosis in mammary gland and cancer. *Endocr Relat Cancer* 2000;7(4):257-69.
29. Krajewski S, Krajewska M, Turner BC, Pratt C, Howard B, Zapata JM, et al. Prognostic significance of apoptosis regulators in breast cancer. *Endocr Relat Cancer* 1999;6(1):29-40.
30. Yu B, Sun X, Shen HY, Gao F, Fan YM, Sun ZJ. Expression of the apoptosis- related genes Bcl-2 and BAD in human breast carcinoma and their associated relationship with chemosensitivity. *J Exper Clin Cancer Res* 2010;29:107-13.
31. Coultas L, Strasser A. The role of the Bcl-2 protein family in cancer. *Semin Cancer Biol* 2003;13(2):115-23.
32. Miyashita T, Kitada S, Krajewski S, Horne WA, Delia D, Reed JC. Overexpression of the Bcl-2 protein increases the half-life of P21 Bax. *J Biol Chem* 1995;270(44):26049-52.
33. Martinez-Arribas F, Martin-Garabato E, Zapardiel I, Sanchez J, Lucas AR, Tejerina A, et al. Bax expression in untreated breast cancer: An immunocytometric study of 225 cases. *Anticancer Res* 2008;28(5A):2595-8.
34. Gaballah HE, Abdel Salam I, Abdel Wahab N, Mansour OM. Plasma Bcl-2 and nitric oxide: Possible prognostic role in patients with metastatic breast cancer. *Med Oncol* 2001;18(3):171-8.
35. Gross A, McDonnell JM, Korsmeyer SJ. Bcl-2 family members and the mitochondria in apoptosis. *Genes*

Dev. 1999;13(15):1899–911.
36. Vargas-Roig LM, Cuello-Carrion FD, Fernandez-Escobar N, Daguerre P, Leuzzi M, Ibarra J, et al. Prognostic value of Bcl-2 in breast cancer patients treated with neoadjuvant anthracycline based chemotherapy. *Mol Oncol* 2008;2(1):102-11.
37. Ruley HE. P53 and response to chemotherapy and radiotherapy. *Principles Pract Oncol* 1997;11:7-19.
38. Michael D, Oren M. The p53-Mdm2 module and the ubiquitin system. *Cancer Biology* 2003;13(1):49-58.
39. Zellars RC, Hilsenbeck SG, Clark GM, Allred DC, Herman TS, Chamness GC, et al. Prognostic value of p53 for local failure in mastectomy treated breast cancer patients. *J Clinl Oncol* 2000;18(9):1906-13.
40. Balogh GA, Mailo DA, Corte MM, Roncoroni P, Nardi H, Vincen E, et al. Mutant p53 protein in serum could be used as a molecular marker in human breast cancer. *Int J Oncol* 2006;28(4):995-1002.
41. Nadasi E, Anga B, Sandor J, Megyesi J, Kelemen D, Mottolese M, et al. Prognostic factor in Hungarian breast cancer patients. *Anticancer Res* 2007;27(1A): 279-82.
42. Bosari S, Lee AK, Viale G, Heatley GJ, Coggi G. Abnormal p53 immunoreactivity and prognosis in node-negative breast carcinomas with long- term follow- up. *Virchows Arch A Pathol Anat Histopathol* 1992;421(4):291-5.
43. Gunther T, Schneider-Stock R, Rys J, Niezabitowaski A, Roessner A. p53 gene mutations and expression of p53 and Mdm2 protein in invasive breast carcinoma A comparative analysis with clinico-pathological factors. *J Cancer Res Clin Oncol* 1997;123(7):388-94.
44. Gunel N, Akcali Z, Coskun U, Akyol G, Yamac D, Yenidunya S. Prognostic importance of tumor angiogenesis in breast carcinoma with adjuvant chemotherapy. *Pathol Res Pract* 2002;198(1):7-12.
45. Allred DC, Gary GM, Elledge R, Fuqua SAW, Brown RW, Chamness GC, et al. Association of p53 protein expression with tumor cell proliferation rate and clinical outcome in node-negative breast cancer. *J Natl Cancer Inst.* 1993;85(3):200-6.
46. Sabbatini P, Chiou SK, Rao L, White E. Modulation of p53-mediated transcriptional repression and apoptosis by the adenovirus E1B 19K protein. *Mol Cell Biol* 1995;15(2):1060-70.
47. Ali AA, Alattar A, Amin M. Prognostic significance of Bcl-2, Bax and p53 expression in breast cancer. *Egypt J Surg* 2006;25(4):213-20.
48. Duffy MJ. Biochemical markers in breast cancer: Which ones are clinically useful? *Clin Biochem* 2001;34(5):347-52.
49. Retz M, Lehmann J, Roder C, Plotz B, Harder J, Eggers J, et al. Differential mucin MUC7 gene expression in invasive bladder carcinoma in contrast to uniform MUC1 and MUC2 gene expression in both normal urothelium and bladder carcinoma. *Cancer Res* 1998;58(24):5662-6.
50. Hewala T, Abd El-Moneim NA, Ebied SA, Sheta MI, Soliman K, Abu-Elenean A. Diagnostic and prognostic value of serum nitric oxide, tumor necrosis factor- α, basic fibroblast growth factor and copper as angiogenic markers in premenopausal breast cancer patients: A case-control study. *Br J Biomed Sci* 2010; 67(4):167-76.
51. Kerin MJ, McAnena OJ, Omalley OP. CA 15-3: Its relationship to clinical stage and progression to metastatic disease in breast cancer. *Br J Surg* 1989;76(8):838-9.
52. Duffy MJ. Serum tumour markers in breast cancer: Are they of clinical value? *Clinical Chemistry* 2006;52(3): 345-51.
53. Tyshler LB, Longton GM, Ellis GK, Livingston RB. False positive tumor markers: Elevation in patients with breast cancer on FAC-type chemotherapy and correlation with the development of hand and foot syndrome. *Int J Biol Markers* 1996;11(4):203-6.
54. Ebeling FG, Stieber P, Untch M, Nagel D, Konecny GE, Schmitt UM, et al. Serum CEA and CA 15-3 as prognostic factors in primary breast cancer. *Br J Cancer* 2002;86(8):1217-22.