Bcl-2 oncoprotein expression in breast cancer, its relation to estrogen and progesterone receptors and other prognostic factors

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ABSTRACT

Aims of the study: To evaluate the expression of the Bcl-2 oncoprotein in patients with primary breast cancer, to correlate it with estrogen and progesterone receptors and various prognostic parameters.

Patients and methods: Fifty two cases of primary breast cancer in which the estrogen and progesterone receptors statuses were previously tested by immuno-histochemical staining, were included in this retrospective study. The cases were collected from Al- Jamhori Teaching Hospital, Nineveh Private Hospital and Private laboratories. The expression of Bcl-2 oncoprotein was evaluated immunohistochemically; the findings were correlated with the estrogen and progesterone receptors, the age of the patients, size, type and grade of the tumor, lymph node status and vascular invasion.

Results: Bcl-2 oncoprotein was detected in 24 cases of primary breast cancer (46.2%). In this study the majority of estrogen and progesterone receptors positive cases, (87.5%) and (83.3%) respectively, showed positive Bcl-2 oncoprotein expression, (P<0.001) and (P=0.0002) respectively. A significant association was found between Bcl-2 and tumor type (P=0.017). Bcl-2 oncoprotein was directly correlated with the age of the patients (P=0.0047), and inversely with the grade of the tumor and vascular invasion (P value = 0.0092, <0.001) respectively. No significant correlation with tumor size nor with lymph node status could be found, (P=0.078) and (P=0.19) respectively.

Conclusions: Bcl-2 oncoprotein was positive in 46.2% of primary breast cancer. This study revealed a significantly direct correlation between the Bcl-2 and the estrogen and progesterone receptors. A significant association was found between Bcl-2 oncoprotein and tumor type. Bcl-2 was directly related to the age of the patients, and inversely related to the grade of the tumor and vascular invasion.

Keywords: Bcl-2 oncoprotein, breast cancer, estrogen receptor, progesterone receptor.

الخلاصة

أهداف الدراسة: تهدف هذه الدراسة الى تقيّم تعبير البروتين السرطاني Bcl-2 لمرضى سرطان الثدي الابتدائي، وإيجاد علاقتها بمُستقبلات الاستروجين والبروجستيرون والمثبتات الانذارية المختلفة.

المرضى وطرق العمل: اثنان وخمسون حالة من حالات سرطان الثدي الأبتدائي التي فيها حالة مُستقبلات الاستروجين ومستقبلات البروجستيرون قد أختبرت َبكيمياء النسيج المناعي سابقاً، تم اختبارها في هذه الدراسةِ ذات الأثر الرجعي، وقد جُمِعتْ الحالات من مستشفى الجمهوري التعليمي ومستشفى نينوى الخاص والمختبرات الخاصّة. قُيّم تعبير البروتين السرطاني Bcl-2 بكيمياء النسيج المناعي وتم تحليل النَتائِج وفقاً لعُمر المرضى و حجم السرطان، نوعه ، درجته و حالة العُقد اللمفاوية، الغزو الوعائي ووجود مستقبلات الاستروجين والبروجستيرون.

النتائج: وجد البروتين السرطاني Bcl-2 في ٢٤ حالة من حالات سرطان الثدي (٤٦,٢%) و ان أغلبية الحالات التي فيها مُستقبلات الاستروجين إيجابية (٨٧,٥%) أظهرت نتيجة ايجابية للبروتين السرطاني Bcl-2 (P <٠,٠٠١) وأيضاً أغلبية

الحالات التي كانت فيها مُستقبلات البروجستيرون إيجابيةِ (٣,٨٣ %) كانت ايجابية لتعبير Bcl-2 (P=٠,٠٠٠٢). وقد وجدت علاقة هامة بين Bcl-2 ونوع السرطان (P= ٠,٠١٧) والبروتين السرطاني Bcl-2 كانت له علاقة مباشرة بعُمر المرضى (P=٠,٠٤٧) وعلاقة عكسية بدرجة الورم والغزو الوعائي (P = ٠,٠٠٩٢، P< ٠,٠٠١ على التوالي). لم يتم الحصول على علاقة هامّة بين Bcl-2 وحجم الورم (P= ٠,٠٧٨). أيضاً لم يظهر البروتين السرطاني Bcl-2 علاقة هامّة مع حالة العُقد اللمفاوية (P=٠,١٩).

الاستنتاجات: البروتين السرطاني Bcl-2 كان إيجابي في ٤٦,٢% من حالات سرطان الثدي الأبتدائي. وقد أظهرت هذه الدراسة علاقة مباشرة وهامة بين Bcl-2 ومستقبلات الاستروجين والبروجستيرون كما وجدت علاقة هامة بين نوع السرطان والبروتين السرطاني Bcl-2. البروتين السرطاني Bcl-2 كانت له علاقة مباشرة بعُمر المرضى وعلاقة عكسية بدرجة الورم وغزو الأوعية.

Bcl-2 is an intracellular membrane-associated protein of 24-kilodalton [1, 2]. It has been localized in the nuclear envelope, endoplasmic reticulum, and outer mitochondrial membranes of hematopoietic and lymphoid cells, neurons, many epithelial cells, and endocrine-influenced glandular epithelium such as the thyroid, prostate, endometrium, and breast [1].

Bcl-2 inhibits apoptosis (antiapoptosis) but, paradoxically, it has antiproliferative effect [3,4]. Cells overexpressing the Bcl-2 gene product, not only showed a delayed onset of apoptosis but also a rapid arrest in the G1 phase of the cell cycle [5]. The over-expression of Bcl-2 in breast cancer has been found to be associated with several favorable prognostic factors such as smaller size, ER and PR positivity, low cell proliferation rate, and low nuclear grade [1,6,7,8]. The Bcl-2 oncoprotein has been shown to be expressed in 40%-80% of breast cancers [9,10,11], and in ER positive cases it is expressed in 80%-90% [11]. It was found that, similar to PR, the Bcl-2 gene itself is ER regulated. Thus, high Bcl-2 may be indicative of an intact ER pathway that is driving tumor growth and should be sensitive to endocrine therapy[12]. It was observed that there is a greater benefit of tamoxifen in ER+/Bcl-2+ patients as opposed to ER+/Bcl-2– one [13].

Patients and methods

Fifty-two cases of primary breast cancer whose ER and PR statuses had been tested by immuno-histochemical technique were included in this retrospective study. The cases were collected from Al-Jamhori Teaching Hospital, Nineveh Private Hospital and other Private Laboratories. Sections from paraffin embedded tissues were taken on clean slides and stained with hematoxylin and eosin, then examined under the light microscope. Histological typing was determined according to WHO classification. Histological grading for invasive ductal carcinoma was performed following Nottingham Modification of the Bloom-Richardson system. Other information regarding the age of the patient, size of tumor, vascular invasion, axillary lymph node involvement, and ER and PR statuses were obtained from the medical records.

The Bcl-2 protein was assessed by immunohistochemical technique. The procedure followed the instruction provided by the manufacturer. The materials for the procedure were obtained from Dakocytomation (Monoclonal Mouse Anti-Human bcl-2 Oncoprotein Clone: 124 Isotype: IgG1, kappa (code N1587), and the Detection system EnVision G|2 System/AP, Rabbit/Mouse (Permanent Red) (code K5355)).

The Bcl-2 positivity was expressed by reddish staining that was always localized to the cytoplasm of malignant epithelial cells. Positive and negative controls were included in each run. Sections from normal tonsillar tissue specimens were used as positive controls and the same tissue was used as negative control but with use of universal negative control instead of primary antibody. Normal ductal epithelial cells of the breast and infiltrating lymphocytes, which always expressed Bcl-2, were used as the internal positive control. Both

the intensity of staining and the percentage of the stained cells were scored. Intensity score range from (0-3) depend on the comparison of the intensity of positive cells to the intensity of positive control, while the proportional score estimate the percentage of tumor cells and it range from (0-4), then multiply both the intensity score with the proportional score to get the total score.

Intensity score (IS)	Intensity score observation
0	None
1	Weak
2	Intermediate
3	Strong

Proportion Score (PS)	Proportional score observation
0	0%
1	< 10%
2	10-50%
3	51-80%
4	> 80%

Total score= PS×IS (range = 0-12), 0-4 -----› Negative, 6-12-----› Positive.

Statistical analysis: Association between Bcl-2 expression and variable categories were assessed using Chi-square test or Fisher Freeman Halton test when indicated, and P value of <0.05 was regarded as statistically significant.

Results

The various clinical and pathological parameters of the patients are shown in the following table.

			No.	%
Age	21-30		5	9.6%
	31-40		9	17.3%
	41-50		14	26.9%
	51-60		9	17.3%
	61-70		12	23.1%
	≥71		3	5.8%
Size	<2cm (T1)		3	5.8%
	2-5 cm (T2)		38	73%
	>5 cm (T3)		11	21.2%
Types	DCIS		1	1.9%
	ILC		4	7.7%
	IDC	IDC (NOS)	43	82.7%
		Medullary	2	3.85%
		Mucinous	2	3.85%
Grading of IDC	I		3	6.4%
	II		22	46.8%
	III		22	46.8%
Lymph node metastasis	Positive		39	75%
	Negative		13	25%
Vascular invasion	Positive		27	51.9%
	Negative		25	48.1%
Estrogen receptor	Positive		28	53.8%
	Negative		24	46.2%
Progesterone receptor	Positive		29	55.8%
	Negative		23	44.2%

Immunohistochemical demonstration of Bcl-2 was positive in 24 cases (46.2%). Positive expression of Bcl-2 was significantly associated with positive ER and PR. The majority of ER positive cases (87.5%) showed Bcl-2 positive (P<0.001) and also the majority of PR positive cases (83.3%) showed positive Bcl-2 (P=0.0002), figures (1 & 2). There was a significant direct correlation between the Bcl-2 and age (P=0.0047), with the largest percentage of Bcl-2 positivity seen in patients with the age group 41-50 years. No statistically significant relation was found between Bcl-2 and the tumor size (P=0.078), figure (3).

Regarding the tumor types, a significant correlation was found (P=0.017). There were 2 cases of Mucinous carcinoma, both of them were positive for Bcl-2, half of the cases of both ILC and medullary carcinoma and 41.9% of IDC (NOS) were positive for Bcl-2. There was one case of DCIS, it was positive too, figure (4).

An inverse significant relation was found between Bcl-2 and the grade of the tumor, the Bcl-2 positive cases decreased from 63.7% in grade II to 22.7% in grade III, while Bcl-2 negative cases increase with the increasing of the grade (P=0.0092), figure (5). The expression of Bcl-2 was inversely correlated with the vascular invasion, among the vascular invasion positive patients, 75% of them were Bcl-2 negative and among those with vascular invasion negative, 75% were Bcl-2 positive (P<0.001), figure (6). No significant correlation could be obtained between Bcl-2 and lymph node metastasis (P=0.19).

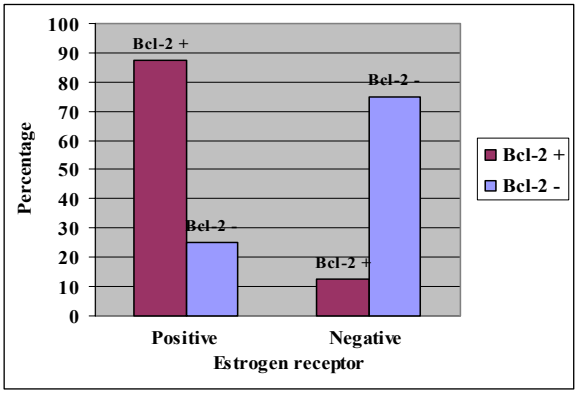

Figure (1): Bcl-2 and estrogen receptor.

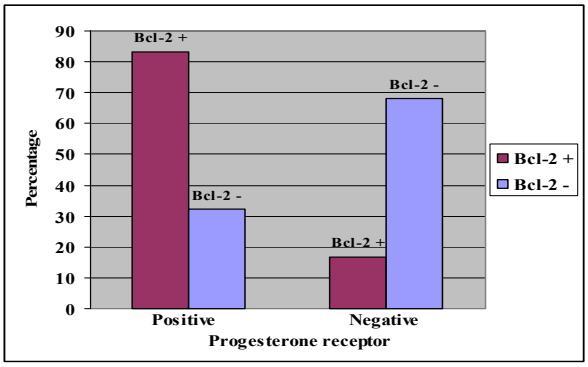

Figure (2): Bcl-2 and progesterone receptor.

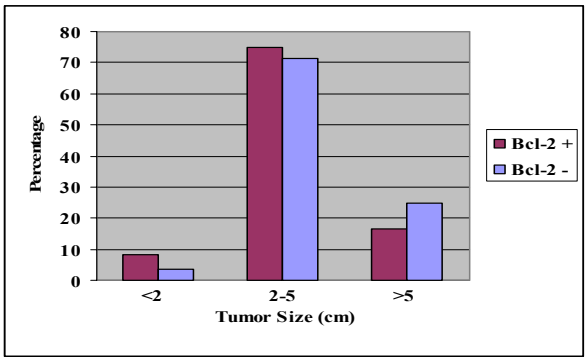

Figure (3): Bcl-2 and tumor size.

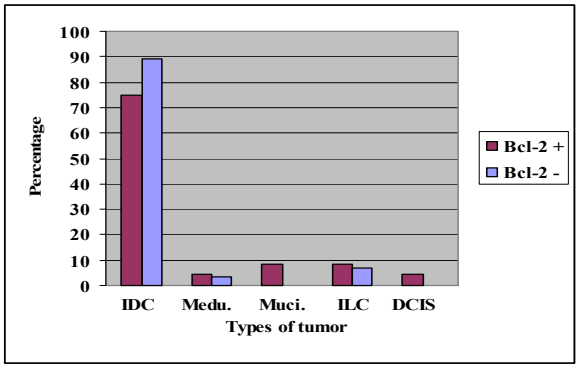

Figure (4): Bcl-2 and the tumor type.

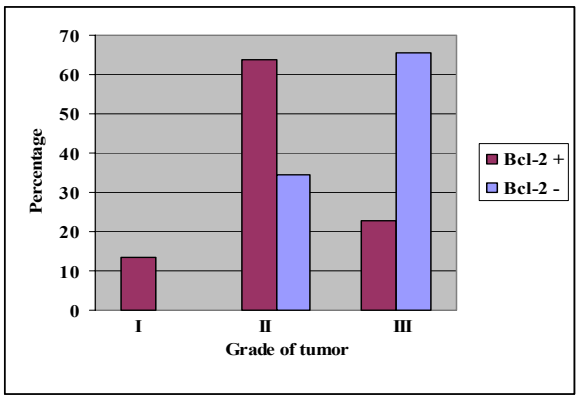

Figure (5): Bcl-2 and the grade of tumor.

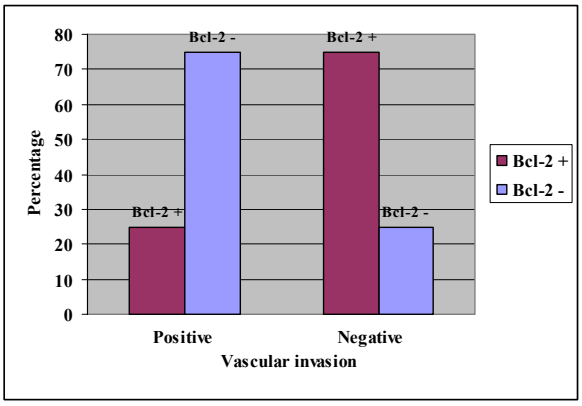

Figure (6): Bcl-2 and vascular invasion.

Discussion

Bcl-2 expression in breast cancer is associated with favorable prognostic factors, and it predicts a good outcome in early breast cancer and even in metastatic disease [14]. Therefore evaluation of Bcl-2 expression in breast cancer may identify a subset of patients with favorable prognosis, who may not benefit from chemotherapy but may benefit from Bcl-2 targeting agents in addition to antihormonal therapy [15].

A strong positive relation between Bcl-2 immunoreactivity and ER and PR statuses was found in this study. This is in agreement with other studies [16-22]. This observation confirms the hypothesis that this protein, like PR, is under oestrogen regulation via ER [1,23]. Binding of estrogen to ER causes its phosphorylation and dimerization followed by transcription of a variety of genes, including secreted growth and angiogenic factors, as well as PR and Bcl-2 [12]. Linke et al in a study of 324 cases of breast cancer found that the disease-specific survival and overall survival of ER-positive patients who were PR negative and who had low Bcl-2 scores were not statistically different from ER-negative patients [12]. However, if either PR was present or the Bcl-2 score was high, the ER-positive patients had significantly better outcome [12]. ER-positive patients who were both PR positive and Bcl-2 high experienced even better outcome [12]. The strong association between Bcl-2, ER, and PR may suggest that co-targeting these molecules in hormone receptor-positive breast cancer might provide greater benefit than chemotherapy, or might play a role as beneficial strategy for sensitizing these tumors to chemotherapy [15].

There is a slight increase of Bcl-2 expression with the increase of the age of the patients, a significant direct correlation was found (p=0.0047). This result is in agreement to that found by Andalib et al [17] and Daidone et al [21]. This may be due to the fact that tumors of elderly patients have a more favorable patho-biological phenotype as to be of well differentiated, low proliferative rate and higher ER content, compared to those of younger age group [21]. This observation is helpful in choosing the method of therapy.

High Bcl-2 expression is associated with small size tumor [6,13]. This can be explained by the inhibitory effect of Bcl-2 on cell proliferation. The acquisition of Bcl-2 expression creates a restrictive environment for the expansion of genetically unstable and potentially malignant cells, causing a delay in tumor progression [6,24]. Although the Bcl-2 positivity in the present study is high in the small tumor size and decreases with the increasing size of tumors, the relation between Bcl-2 and the size failed to reach the significant level (p=0.078). This may be due to the small size of the sample which contains few small size tumor; in fact the percentage of small size tumor in this study was 5.8% <2cm. This is in agreement with Tustusi et al [5], Li Zhang et al [16] and Kumravel et al [25].

In the present study, a significant relationship between Bcl-2 and breast cancer types (p=0.0171) was found. This is comparable to those found by Joensuu et al [26]. On the other hand Coppola et al [27] in their study found no significant relation between Bcl-2 and histological types of breast cancer. This difference may be again due to the small number of cases included in their study (26 cases).

A significant inverse relationship between expression of Bcl-2 protein and grading of tumor was reported. Similar association was noticed by Tsutsui et al [5], Callagy et al [7], and others [6,13,20,28]; this may be due to role of Bcl-2 during the early stage of the tumor as it rescues cells with otherwise lethal mutations

[29]. But after additional oncogene activation, some cells would acquire additional ways to protect themselves against apoptosis [29]. At this point, loss of Bcl-2 might confer a growth advantage. In fact, Bcl-2 is known to restrain cell proliferation. Thus, expression of Bcl-2 would change from high levels in early or low-grade tumors, characterized by low apoptotic indices, to low levels in advanced or high-grade tumors, characterized by high apoptotic indices [29].

A significant inverse relation was obtained between Bcl-2 and LVI. A high Bcl-2 positivity was observed in the tumors without LVI compared to those with LVI. This finding is similar to that of Neri et al [22].

No significant relation could be obtained between Bcl-2 and axillary lymph node status in this study, this result is similar to what had been found by Tsutsui et al [5], Callagy et al [7], Kumravel et al [25], Joensuu et al [26] and Zhang et al [28]. However, Chen et al [20] in a study done on 74 cases of breast cancer found, Bcl-2 immunoactivity to be significantly correlated with negative axillary lymph node and in cases with less nodal involvement.

A number of studies found that the Bcl-2 is an independent prognostic factor in breast cancer [7,14,30]. Morever other studies found that an independent favorable prognostic impact of Bcl-2 is particularly significant among LN+ patients, and the prognostic value of Bcl-2 staining among all patients is solely based on prognostic applicability among LN+ patients because the association among LN- patients is clearly not significant [31]. Accordingly further studies may be needed on larger sample of cases.

Conclusions

Bcl-2 is frequently expressed in breast cancer. A significant direct association has been observed between Bcl-2 oncoprotein and hormonal receptors (ER & PR).

The expression of Bcl-2 is directly correlated with the age of patients, inversely correlated with the grade of tumor and vascular invasion.

A significant association was found between Bcl-2 and tumor type. The Bcl-2 has no relation with the tumor size and axillary lymph node metastasis.

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