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Immunohistochemical Expression of B-Cell Lymphoma-2 Gene with Clinicopathological Correlation: A Comparative Analysis of Triple Negative and Non-Triple Negative Breast Cancers

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ABSTRACT

Background: B-Cell Lymphoma-2 gene is an anti-apoptotic protein associated with favorable prognosis in patients with breast cancer. The present study focused on immunohistochemical expression of the aforementioned gene with clinicopathological correlation in non-triple negative breast cancers (NTNBCs) and triple negative breast cancers (TNBCs).

Methods: We investigated 258 cases of primary breast cancers; they were divided into 2 groups (NTNBCs and TNBCs) based on their expression of estrogen receptor (ER), progesterone receptors (PR), and Her-2/neu receptors. BCL-2 expression was correlated with age, tumor size, tumor grade, histological subtype, lymph node status, and lymphovascular invasion (LVI).

Results: Among NTNBCs, 68.2% of cases expressed BCL-2 as compared to 53.3% in TNBCs (P-value = 0.035). In both groups, BCL-2 expression was significantly higher in younger patients, without lymphovascular invasion and lower grade (borderline significant in TNBCs). The variable which was associated with higher BCL-2 expression only in NTNBCs was smaller tumor size. In contrast, in TNBCs invasive ductal carcinoma was significantly associated with BCL-2 expression.

Conclusions: BCL-2 showed association with various clinicohistopathological characteristics in TNBC and NTNBC breast cancer patients. Yet, some of these variables were potentially related to better prognosis (the lack of LVI, smaller tumor size, and lower grade). On the other hand, younger age, which is a feature generally associated with poorer prognosis, was significantly related to BCL-2 expression.

Introduction

Breast carcinoma is the most popular cancer affecting the females, worldwide. Globally, every year, 1.4 million new cases of breast cancer are diagnosed.[1] Breast cancer incidence varies based on different regions of the world.

This difference is related to differences such as race, genetics, cultural differences, and environmental exposures in various parts of the world.[2] Some studies conducted in India and Pakistan showed increase in the incidence of breast cancer, which were mostly ER, PR negative.[2]

Moreover, this incidence has been shown to be increased during past decade. According to Karachi Cancer Data, the incidence of breast cancer

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increased from1995 to 1995 by 53.1% and more prominently as high as 69.1% from 1998 to 2002 in Asia.[3]

The patients with breast cancer, who lack the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (Her-2/neu), are called triple negative breast cancers (TNBCs).[4] Triple negative breast cancers account for 10% to 20% of all patients with breast cancer. The patients with breast cancer, who can express either the hormone receptors ER, PR, or Her-2/neu, are non-triple negative breast cancers (NTNBCs). Most of the TNBCs, but not all of them, are basal type on gene expression profiling.

The TNBCs have an aggressive behavior,[5] increased chance of metastasis,[6,7] and unfavorable prognosis as compared to NTNBCs.

Some prognostic and predictive elements have extensively been studied in patients with breast cancer. B-cell lymphoma-2 (BCL-2) is a significant prognostic indicator in patients with breast cancer, and its expression, likewise, can be used as targeted therapy for some new drugs.[8]

BCL-2 is an anti-apoptotic protein expressed in 25% to 50% of patients with breast cancer.[9,10] BCL-2, in some studies, was correlated with progressed rate of survival, and it was reported as a favorable prognostic factor.[10-14] On the contrary, the lack of BCL-2 expression was shown to be an independent indicator of poor prognosis.

The number of studies showing prognostic importance of BCL-2 expression in TNBCs and NTNBCs is very limited. Keeping in view its prognostic significance, it has been suggested in various studies to include BCL-2 expression in the Nottingham Prognostic Index.

The aim of the present study was to compare the expression of BCL-2 in TNBCs and NTNBCs as well as to correlate its expression with various clinicopathological parameters. The present study would help to improve patients' stratification and to provide clinicians with more management criteria.

Methods

This cross-sectional study was conducted at Histopathology Department of Armed Forces Institute of Pathology, Rawalpindi, Pakistan. A total of 258 primary patients with breast carcinomas were diagnosed from 20th January 2014 to 20th July 2016; they were included since hormone receptor and Her-2 status was available.

Age and other histopathologic parameters, such as histologic grade, type, nuclear grade, and lymphovascular invasion (LVI) were obtained from patient pathology records. Nottingham Criteria of Bloom & Richardson was used for grading all the tumors.[15]

Immunohistochemistry (IHC) was applied on the most representatives and well preserved areas of tissue blocks fixed with 10% buffered formalin. The results of IHC were evaluated and scored semi-quantitatively by 2 histopathologists.[16] A cut off value was given for each marker for positive or negative staining. Hormone receptors were taken as positive if at least 1% of tumor cells showed moderate to strong nuclear staining.[17]

For Her-2/neu, membrane staining would be scored 0, if there was no or faint incomplete staining in <10% cells; 1, if there was faint incomplete staining in >10% cells. Score 3 was perceived positive with complete chicken wire like staining in >10% cells.[16,17]

For BCL-2, cytoplasmic or membranous staining of 10% or more tumor cells were perceived positive.[15] Cases with moderate to strong staining for this marker was regarded as positive as shown in figure 1.[18] IHC analyses for BCL-2 was performed on all specimens.

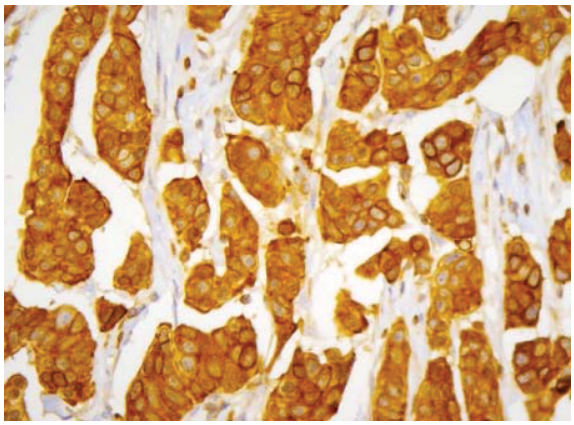

Figure 1. Cytoplasmic positivity of BCL-2 protein

Statistical analysis was performed using SPSS version 19.0. The descriptive statistics were calculated for both quantitative and qualitative variables. Chi square test was used for analysis. A P-value of less than 0.05 was considered as statistically significant.

Results

A total of 258 patients were included in the present study. They consisted of 217 (84%) invasive ductal carcinomas, 24 (9.3%) invasive lobular carcinomas, and 6 (2.3%) mixed ductal and lobular carcinomas. The others included 3 (1.2%) mucinous, 3 (1.2%) tubular, 2 (0.8%) papillary carcinomas, 2 (0.8%) metaplastic, and 1 (0.4%) medullary carcinoma. The patients' age ranged from 28 to 90 with the mean age of 49. All the patients were females. Out of 258 cases, 198 were NTNBCs and 60 were TNBCs.

BCL-2 expression and its further correlation with clinicopathological parameters were summarized in table 1 and table 2.

Table 1. Correlation of BCL-2 expression and clinicopathological variables in non-triple negative breast cancers (N = 198)

Clinicopathological variables	Cases (N)	Percentage (%)	BCL-2 Expression		P-value
			Positive	Negative	
Patients' age					0.01
< 50 Years	94	47.5	72 (76.6%)	22 (23.4%)	
≥ 50 Years	104	52.5	63 (60.6%)	41 (39.4%)	
Tumor Size					0.002
<2 cm	29	14.7	22 (76%)	7 (24%)	
2-5 cm	108	54.5	82 (75%)	26 (25%)	
>5cm	61	30.8	31 (51%)	30 (49%)	
Histologic Type					0.68
IDC	164	82.9	110 (67%)	54 (33%)	
ILC	20	10.1	16 (80%)	4 (20%)	
Mixed	6	3	4 (66.7%)	2 (33.3%)	
Others	8	4	5 (62.5%)	3 (37.5%)	
Tumor Grade					0.001
Grade I	3	1.5	3 (100%)	0	
Grade II	153	77.3	112 (73.2%)	41 (26.8%)	
Grade III	36	18.2	16 (44.4%)	20 (55.6%)	
Not applicable	6	3	4 (66.7%)	2 (33.3%)	
Lymph Node Metastasis					0.26
Seen	119	60.1	78 (65.5%)	41 (34.5%)	
Not Seen	61	30.8	45 (73.8%)	16 (26.2%)	
Not Identified	18	9.1	12 (66.7%)	6 (33.3%)	
Lymphovascular Invasion					<0.002
Seen	94	47.5	54 (57.4%)	40 (42.6%)	
Not Seen	104	52.5	81 (77.9%)	23 (22.1%)	

Table 2. Correlation of BCL-2 expression and clinicopathological variables in triple negative breast cancers (N=60)

Clinicopathological variables	Cases (N)	Percentage (%)	BCL-2 Expression		P-value
			Positive	Negative	
Patients' age					0.02
< 50 Years	35	58.3	23 (65.7%)	12 (34.3%)	
≥ 50 Years	25	41.7	9 (36%)	16 (64%)	
Tumor Size					0.12
<2 cm	7	11.7	4 (57%)	3 (43%)	
2-5 cm	33	55	21 (63.6%)	12 (36.4%)	
> 5cm	20	33.3	7 (35%)	13 (65%)	
Histologic Type					0.07
IDC	53	88.3	31 (58.5%)	22 (41.5%)	
ILC	4	6.7	1 (25%)	3 (75%)	
Mixed	0	0	0	0	
Others	3	5	0	3 (100%)	
Tumor Grade					0.05
Grade I	1	1.6	1(100%)	0	
Grade II	41	68.3	26 (63.4%)	15 (36.6%)	
Grade III	16	26.7	5 (31.3%)	11 (68.7%)	
Not applicable	2	3.4	0	2 (100%)	
Lymph Node Mets					0.10
Seen	37	61.7	16 (43.2%)	21 (56.8%)	
Not Seen	18	30	12 (66.7%)	6 (33.3%)	
Not Identified	5	8.3	4 (80%)	1 (20%)	
Lymphovascular Invasion					0.01
Seen	24	40	8 (33.3%)	16 (66.7%)	
Not Seen	36	60	24 (66.7%)	12 (33.3%)	

BCL-2 expression was more common in patients aged less than 50 as compared to those with age more than 50 in both NTNBCs and TNBCs, and it was statistically significant with P-value of less than 0.05. There was higher expression of BCL-2 in tumors smaller than 5 cm, but the expression decreased in tumors more than 5 cm (51%). It was statistically significant (as shown in table 1), but such significance was not found in TNBCs. BCL-2 expression was higher (80%) in invasive lobular cancers as compared to invasive ductal carcinomas (67%) in NTNBCs, but its expression was higher in ductal carcinomas in TNBCs.

There was also an inverse relationship of BCL-2 expression with grade of tumor being higher in low grade tumors as compared to high grade tumors in both NTNBCs and TNBCs with P-value less than 0.05. Tumors with BCL-2 expression showed lower incidence of lymph node metastasis and LVI in both NTNBCs and TNBCs, which was statistically significant, as shown in table1 and table 2.

Regarding the association between BCL-2 expression and TNBCs/NTNBCs, BCL-2 expression was observed in 32 out of 60 TNBCs (53.3%), while it was detected in 135 out of 198 NTNBCs (66.2%). This difference was statistically significant (Chi square = 4.45, P-value = 0.035).

Discussion

There are different proteins, which regulate cell cycle, involved in initiation, progression, and treatment response in various malignancies. Some studies have recently been performed to detect various genes and proteins involved in regulation of apoptosis.

BCL-2, an anti-apoptotic gene, is extensively examined in patients with breast cancer for disease progression, treatment response, radiation therapy, chemotherapy, and more recently targeted therapy.[8] It was previously proved that follicular lymphomas as anti-apoptotic protein was associated with worse outcome, but in patients with breast cancer, it was associated with favorable outcomes.[19]

Considering its prognostic and therapeutic importance, we have compared BCL-2 expression of NTNBCs and TNBCs immunohistochemically.

The results of the previous research have revealed that BCL-2 was associated with better prognosis in all types of breast cancers, correlating with previous studies, as well.[11, 20-22] It was also associated with disease-free survival and increased overall survival.[10] The study revealed that BCL-2 expression was directly associated with ER and PR expression, and inversely associated with Her-2/neu expression, correlating with previously published studies.[13,23]

Significant association of BCL-2 expression was found with age, which was compatible with the results of the study conducted by Thomadaki *et al.*[24]

BCL-2 expression was significantly associated with tumor size in NTNBCs, but the finding was less significant in TNBCs, which is in line with some international studies.[16] Some other studies showed no relationship between size of tumor and BCL-2 expression.[14,25]

In the present study, poorly differentiated tumors showed less BCL-2 expression than low grade breast cancers in both TNBC and NTNBCs. This finding was similar with the results of the study conducted by Bhargava *et al.* and Park *et al.*[18, 26] It suggested that higher tumor grade was associated with the loss of BCL-2 expression.[12]

Lymph node metastasis has been shown as the most important prognostic and predictive factor in breast cancer along with LVI.[27] In our study, BCL-2 expression was more common in tumors without LVI. This was similar with the findings of another international study.[28]

As was already demonstrated,[12] in our study, TNBCs breast cancer was associated with less BCL-2 expression than NTBCs tumors.

The limitations of the study included the lack of access to follow up of the patients and relatively smaller sample size due to which multivariate analysis could not be performed.

TNBCs showed some differences in BCL-2 expression as compared to NTNBCs. These might be due to BCL-2 participation in various biological pathways with different subsequent effects on cell survival, which was further dependent on tumor phenotype. It is of importance if we can identify different prognostic groups in TNBCs, which one can benefit from newer lines of therapy. BCL-2 expression may be of importance in response to neo-adjuvant chemotherapy in TNBCs.[29]

Such a large scale study has not already been performed in this region. Hence, this study will provide clinicians with a good insight for using BCL-2 as a part of prognostic workup; likewise, its high expression in both types of tumors will incite further workup for targeted therapy against BCL-2.

The present study is one of the few studies examining the prognostic role of BCL-2 in breast cancer in NTNBCs and TNBCs. BCL-2 can be combined to the well-accepted clinicohistopathologic prognostic factors to predict overall prognosis of invasive breast cancer and guide treatment. BCL-2 showed association with various clinicohistopathological characteristics in TNBC and NTNBC breast cancer patients.

Nevertheless, some of these variables were potentially related with better prognosis (lack of LVI, less tumor size and lower grade). On the other hand, younger age, which is a characteristic generally associated with poorer prognosis, was significantly related to BCL-2 expression.

Further research is necessary to elucidate this association with sample size in different sub-groups and follow up. Moreover, targeted therapies against

BCL-2, (like BH3 mimetics ABT-737 & ABT199)[8] which are currently in clinical trials, can be used in future, especially in TNBCs, where limited options are available for treatment.

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