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Original Article

Expression of B-cell Lymphoma 2 in Breast Cancer

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Abstract

Introduction: Immunohistochemical expression of B-cell lymphoma 2 (BCL-2) is seen variably in invasive ductal carcinoma. This study was conducted to determine the frequency of BCL-2 expression in different histologic grades of invasive ductal carcinoma. **Materials and Methods:** A cross-sectional study was conducted in the Department of Pathology at Shaukat Khanum Memorial Cancer Hospital and Research Centre, Pakistan, on subjects with invasive ductal carcinoma of various histologic grades. Immunohistochemistry was done using the BCL-2 antibody in all cases. The frequency of BCL-2-positive cases in different histologic grades was noted. Post-stratification, the Chi-square test was applied. $P \leq 0.05$ was considered statistically significant. **Results:** All 52 subjects were female (100%) with a mean age of 47.58 ± 1.43 years. BCL-2 expression was observed in 28 (53.85 %) subjects with breast cancer. Out of 33 participants with Grade III, 13 (39.39 %) participants were positive for BCL-2 expression. Among 18 subjects with Grade II, 14 (77.78 %) subjects were positive for BCL-2 expression. Reduced frequency of BCL-2 expression was observed with increasing histologic grade (i.e., more in low-grade tumours and less in Grade III), but the difference was statistically not significant. **Conclusion:** A differential expression of BCL-2 was observed across different grades of invasive ductal carcinoma. However, the difference was not statistically significant.

Key words: B-cell lymphoma-2, breast cancer, histologic grades, immunohistochemistry, invasive ductal carcinoma

Introduction

According to the World Health Organisation, breast cancer is the most common malignancy in women worldwide. It affects 2.1 million women every year. In 2018, breast cancer accounted for almost 15% of cancer-associated mortalities. The incidence is higher in developed countries and is increasing globally.[1] Among Asian countries, Pakistani women are next to Israeli Jews in developing breast cancer, accounting for 34.6% of female malignancies.[2] Breast cancer is a heterogeneous disease with a wide variation in morphological features, grades, hormone receptor status and molecular genetics.[3] Different

etiological factors contribute to the development of breast cancer. However, among these, the three major causative factors in breast cancer development are genetic factors, hormonal influences and environmental factors. Almost 40% of hereditary breast carcinomas are associated with BRCA1/BRCA2 gene mutations.[4] Among the various subtypes of breast carcinoma, invasive ductal carcinoma is the most frequently diagnosed subtype.[5]

The process of carcinogenesis is the result of the accumulation of genetic variants that play a role in cell proliferation and apoptosis, thus deciding the fate of the lesion.[6] B-cell lymphoma 2 (BCL-2) protein is a member of the BCL family that regulates apoptosis. Its oncogenic role is supported by its increased expression in lymphomas and various tumours, including breast cancer.[7]

In a study conducted by Ruibal *et al*., higher expression of BCL-2 is seen in Grade I and II tumours of invasive ductal carcinoma than Grade III tumours.[8] A large study conducted by Dawson *et al*. demonstrated BCL-2 overexpression in 73% of breast cancer and served as an independent prognostic factor.[9] In another study carried out in China, BCL-2 expression was noted in 84.61% of Grade I breast cancers, 58.69% in Grade II of breast cancer and 12.50% in Grade III of breast cancer.[10]

The rationale of this study was to determine the expression of BCL-2 in different histologic grades of breast cancer in Pakistani women as BCL-2 is a prognostic marker as well as a potential target for new therapies.[11]

Materials and Methods

A cross-sectional study was conducted in the Department of Pathology at Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, Pakistan. The Institutional Review Board (IRB) approved a waiver of informed consent for this study (EX-25-10-19-01). Fifty-two patients with invasive ductal carcinoma were selected by non-probability consecutive sampling for 5 months extending from 1 December 2017 to 30 April 2018. Patients diagnosed with invasive ductal carcinoma of breast on incisional biopsy, excisional biopsy or mastectomy specimens were included in the study. Poorly fixed samples, scanty biopsies or post-therapy biopsies were not included.

Patient demographics as age and gender, the grade of breast cancer, were recorded. Histological grading was done blindly by two experienced pathologists with a special interest in breast pathology, as per Nottingham's-Bloom-Richardson System modification. It assesses three parameters that is, tubule formation, nuclear pleomorphism and mitotic count, giving a score of 1–3 for each. A total score out of 9 is used to ascertain the grade of the tumour. BCL-2 immunostaining was performed on sections of paraffin blocks using Roche BCL-2 antibody, clone 124. Tissue sections were taken on a glass slide and subjected to Ventana automated immunohistochemistry analyser (Benchmark ultra). Results of immunohistochemistry were interpreted and approved by the consultant pathologists independently to avoid bias. BCL-2 demonstrated cytoplasmic and/or membranous staining of the cells. The cutoff value was taken at 10%, cases with <10% tumour cells staining with BCL-2 or without any staining were considered negative while staining of more than or equal to 10% tumour cells was taken as positive.[12]

Statistical analysis was performed using SPSS version 20 software (SPSS Inc., Chicago, IL, USA). Frequency and percentage were calculated for gender, expression of BCL-2 and grades of breast cancer. Mean ± standard deviation was calculated for qualitative data. Age and tumour grades effect were checked using stratification. The post-stratification Chi-square test was applied to study the significance. $P \leq 0.05$ was considered to be statistically significant.

Results

Fifty-two female subjects with invasive ductal carcinoma of different grades were included in the study. The mean age of the participants was

47.58 ± 1.43 years. Most of the subjects (32.69 %) fell in the age range of 41–50 years [Table 1]. Out of 52 subjects that were included in this study, there was 1 (1.92%) subject with Grade I breast cancer, 18 (34.62%) subjects with Grade II breast cancer and 33 (63.46%) subjects with Grade III breast cancer [Figure 1]. On the evaluation of BCL-2 by immunohistochemistry, 28 (53.85%) cases showed positive BCL-2 expression and 24 (46.15%) cases either did not show any staining for BCL-2 or stained <10% of the tumour cells [Figure 2]. These samples were considered to be negative. Regarding the expression of BCL-2 in different histologic grades of invasive ductal carcinoma, Grade I breast cancer exhibited diffuse expression of BCL-2 in tumour cells. Out of 18 Grade II invasive ductal breast carcinoma, 14 (77.78%) tumours expressed BCL-2, while 4 (22.22%) were negative for BCL-2 expression. Among Grade III tumours, 20 (60.61%) were negative for BCL-2 expression and 13 (39.39%) showed positive BCL-2 expression [Figures 3 and 4].

The post-stratification Chi-square test of significance suggested that the differences in the expression of BCL-2 in different grades of breast carcinoma were statistically not significant ($P = 0.06$) [Table 2]. The highest expression of BCL-2 was seen in the 41–50 years age group (58.82%) and the lowest expression of BCL-2 was found in the 21–30 years age group [Table 3].

Table 1: Distribution of subjects by age (*n*=52)

Age (years)	Number of subjects (*n*)	Percentage (%)
20–30	3	5.77
31–40	13	25
41–50	17	32.69
51–60	16	30.77
61–70	3	5.77
71–80	0	0
Mean±standard deviation	47.58±1.43 years	
Range	25–75 years	

Table 2: Stratification of the cohort by B-cell lymphoma (BCL-2) expression and the grades of breast cancer (*n*=52)

Grades of breast cancer	BCL-2 expression			
	Positive		Negative	
	No.	%	No.	%
I (*n*=1)	1	100	0	0
II (*n*=18)	14	77.78	4	22.22
III (*n*=33)	13	39.39	20	60.61
P-value	0.06			

Table 3: Stratification of the cohort by B-cell lymphoma (BCL-2) expression and age (*n*=52)

Age groups (years)	BCL-2 expression			
	Positive		Negative	
	No.	%	No.	%
21–30 (*n*=3)	1	33.33	2	66.67
31–40 (*n*=13)	6	46.15	7	53.85
41–50 (*n*=17)	10	58.82	7	41.18
51–60 (*n*=16)	8	50	8	50
61–70 (*n*=3)	3	100	0	0
P-value	0.46			

Discussion

Prognosis of breast cancer is dependent on various factors including grade, stage and hormone receptor status.[13] The expression of BCL-2 in breast cancer and its correlation with different breast cancer parameters that is, histologic grade, stage, receptor status and its role as an independent prognostic marker, has been studied in various parts of the world. Literature has shown variable results among different studies. This study aimed to determine the expression of BCL-2 in different histologic grades of breast cancer in Pakistani women.

The current study showed that BCL-2 expression was positive in almost half of the population under investigation (53.85%). This is in concordance with the research carried out by Eom *et al*., in which BCL-2 expression was observed in 53.8% of cases.[12] Similarly, Mdzin *et al*. reported BCL-2-positive expression in 40.7% of cohort.[14] The study also concluded that BCL-2 was expressed in normal breast epithelium adjacent to the tumour,

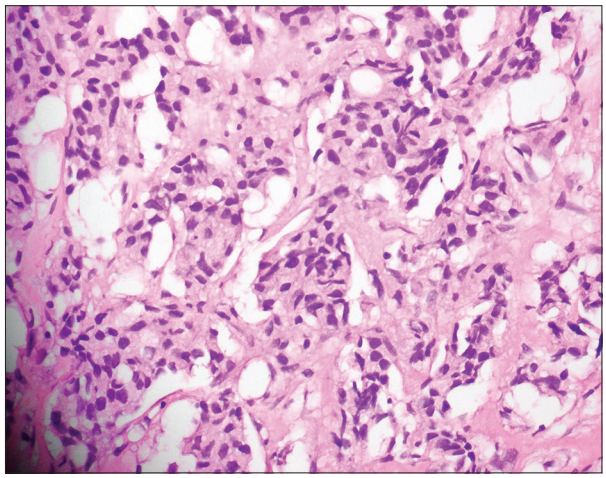

Figure 1: Hematoxylin and eosin (H and E) stained slide of invasive ductal carcinoma Grade II, ×400

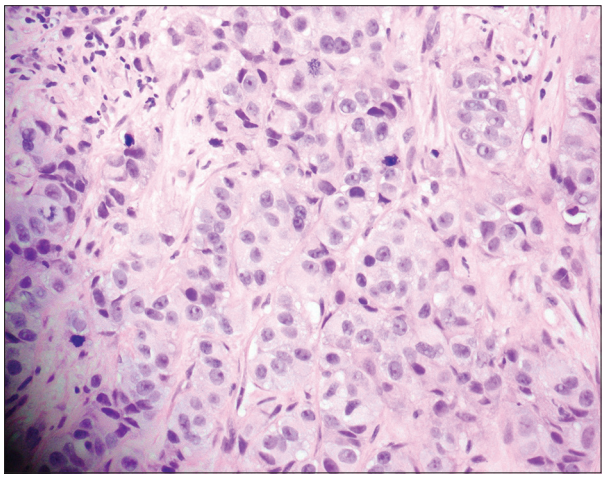

Figure 3: Hematoxylin and eosin (H and E) stained slide of invasive ductal carcinoma Grade III, ×400

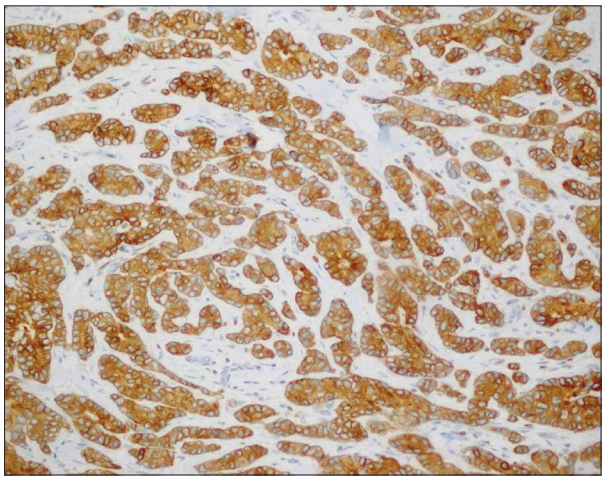

Figure 2: Positive staining of B-cell lymphoma-2 (cytoplasmic and membranous) in invasive ductal carcinoma Grade II, ×200

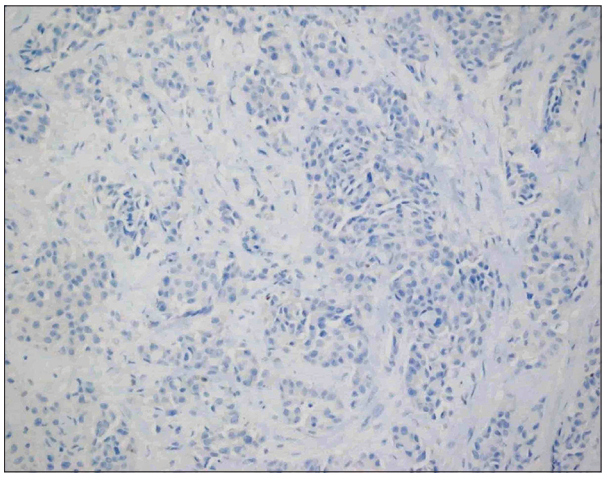

Figure 4: Negative staining for B-cell lymphoma-2 in invasive ductal carcinoma Grade III, ×200

suggesting that BCL-2 expression protects normal epithelial cells from undergoing apoptosis and increases their proliferation.

In a study carried by Joensuu *et al*.,[15] 25% of the subjects showed no immunoreactivity for BCL-2 protein in malignant epithelial cells, 29% had weak expression (+) and 29% showed moderate staining (++). In comparison, 17% exhibited strong staining (+++) for BCL-2 protein. Overall, 75% of subjects were positive for BCL-2 expression. In a study by Dawson *et al*., BCL-2 expressions were seen among 73% of subjects and were found to be a predictor of better prognosis.[9] It was suggested by Mdzin *et al*. that the observed variation of BCL-2 expression in different researches might be attributed to sampling technique, the difference in analysis and the cutoff value.[14]

Since BCL-2 blocks programmed cell death (apoptosis) and contributes to the accumulation of malignant cells, its overexpression raises the possibility of aggressive tumour behaviour. Genetic alteration of the BCL-2 gene, present on chromosome 18, is crucial in developing

chemoresistant neoplasms as follicular lymphoma. However, in breast cancer, positive BCL-2 expression is associated with better differentiation that is, lower grades, low proliferation index and ER positivity.[16] One of these attributes of BCL-2 that is, more expression in low-grade tumours than high-grade tumours, was observed in our study. The majority of the participants belonged to histologic Grades III and II (63.46% and 34.62%, respectively). A different frequency of histologic grading of the subjects had been observed by Dawson *et al*. who document that frequency of Grades I–III was 14%, 41% and 45%, respectively.[9]

In this study, the single Grade I breast cancer demonstrated strong diffuse BCL-2 expression, while Grade II and III cancers expressed BCL-2 with a frequency of 77.78% and 39.39%, respectively ($P > 0.05$). In a study by Yu *et al*., the expression of BCL-2 across Grades I–III of ductal carcinoma was 84.61%, 58.69% and 12.50%, respectively, and the difference was statistically significant ($P < 0.01$).[9] However, Mdzin *et al*. reported that the difference in the BCL-2 expression in Grades I–III was statistically not significant. In a study by Lee *et al*. which was conducted to determine the reciprocal expression of BCL-2 and p53 in ductal carcinoma, it was found that the BCL-2+/p53- pattern predominates in histological Grade I (77.4%) and Grade II tumours (59.3%) with a rarity of BCL-2 expression in Grade III carcinomas (6.3%).[17] Conversely, BCL-2-/p53+ expression was observed in 50% of Grade III carcinomas, while 3.2% and 11.1% expression were noted in Grade I and II tumours, respectively. This pattern of BCL-2/p53 expression by tumours might help in depicting the response to therapy and prognosis. It was also established that BCL-2 expression correlates with other prognostic factors, and its expression could be oestrogen dependent.

Mdzin *et al*. have observed a decrease in BCL-2 expression with a progressive increase in histopathologic grades.[14] We also observed a declining pattern of BCL-2 expression with increasing grades of the tumour, but the results were not significant.

On stratification of data with respect to different age groups, the BCL-2 expression was highest among the participants in the fourth and fifth decades of life. It was the least among subjects in the second and sixth decades of life. However, this difference was statistically not significant $P > 0.05$). Similar differences in BCL-2 expression within different age groups were also observed by Yu *et al*.[18] Contrary to the findings of the present study, the difference was found to be statistically significant ($P < 0.01$). They also found that in menopausal women with breast cancer, the positive expression of BCL-2 was calculated at 88.9% in Grade I tumours, 73.7% in Grade II tumours and 0.0% in Grade III tumours ($P > 0.05$). Therefore, a declining trend of BCL-2 expression with an increase in histological grade was noted.

This study has certain limitations. All of the subjects in the present study were female. This was primarily due to the rarity of invasive ductal carcinomas among males. Other investigators have encountered this issue as well, and because of this reason, the findings of the present study cannot be generalised for both sexes.[15] Similarly, there was only one case of Grade I breast carcinoma. This study was conducted at a single centre in Pakistan, which may raise doubts about the generalisability of the results. However, this is a tertiary care facility, which receives patients from all across Pakistan. The pathologist grading the samples did not undergo reliability assessment, and they were not blind to their colleagues' reviews. Ideally, at least two highly experienced or dedicated breast pathologists should do grading blindly and achieve consensus or exclude those cases in which discrepancy exists to minimise bias. Nonetheless, in this study, grading was done independently by two pathologists with experience of more than 20 years in histopathology. Further studies with larger and more diverse sample sizes are required to study the relationship between grades of breast cancer and BCL-2 expression.

Approximately half (50%) of the study population with invasive ductal carcinoma expressed BCL-2. Although the reduced frequency of BCL-2 expression was observed with increasing histologic

grade (i.e., more in low-grade tumours and less in Grade III tumours), this was not statistically significant.

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Authorship Contributions

Conceived and designed the analysis; SB, ST, AL and SM. Collected the data; SB and ST. Contributed data or analysis tools; AL, SM, UH and MH. Performed the analysis; AL, SM, UH and MH. Wrote the paper; SB, AL, ST, SM, UH and MH.