ORIGINAL ARTICLE

IMMUNOHISTOCHEMICAL EXPRESSION OF BCL2 AND ITS ASSOCIATION WITH OTHER PROGNOSTIC MARKERS IN INVASIVE CARCINOMA OF BREAST, NO SPECIAL TYPE IN TERTIARY CARE HOSPITAL

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Background: B-cell lymphoma 2 (BCL2) is expressed by different malignancies including invasive breast carcinoma, No specific type. In breast carcinoma, its immune-expression is associated with the grade of the tumour and other prognostic factors like Oestrogen Receptors (ER), Progesterone Receptors (PR), Her 2, ki 67 and molecular subtypes. The objective was to determine the association of BCL2 immuno-expression with variable prognostic factors of invasive breast carcinoma. **Methods:** Over-all 71 cases of invasive breast carcinoma, no special type was included and BCL2 expression was evaluated along its association in terms of frequency with hormone receptors (ER, PR) Her2, ki 67 and grade of tumour. The expression of bcl2 was also further evaluated among molecular subtypes of breast carcinoma including luminal A, luminal B, Her 2 enriched and basal like (triple negative). **Results:** Positive Bcl2 expression was noted in 61% of cases of luminal A & B subtypes (hormone responsive tumours) pointing for its significant correlation with them while its relation with grade of the tumour, proliferation index and Her 2 status was found insignificant. **Conclusion:** Expression of BCL2 in invasive breast carcinoma, no special type is considered as a favourable prognostic marker irrespective of its histological grade and proliferation index.

Keywords: B-cell lymphoma 2; Invasive breast carcinoma; Hormone receptors

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INTRODUCTION

Breast carcinoma is the 5th leading source of cancer-related deaths, internationally and responsible for 685,000 deaths in 2020.[1]

Among the various mechanisms involved in carcinogenesis, apoptosis plays a vital part in regulating cellular proliferation and its dysregulation. The B-cell Lymphoma 2 (BCL-2) family of proteins is integral to the regulation of apoptosis, encompassing both pro-apoptotic and anti-apoptotic factors that maintain a critical balance between cell death and the proliferation of malignant clones. Anti-apoptotic proteins within this family include BCL-2, BCL-XL, BCL-W, BCL-B (BCL2L10), MCL-1L, MCL-1, and BFL-1/A1, among others.[2] In contrast, pro-apoptotic proteins consist of BIM, BID, Puma, Mule, BAD, Noxa, BIK/BLK, BMF, HRK/DP5, and Beclin-1.[3]

BCL2 is antiapoptotic factor which inhibits apoptosis and thus its increased expression is associated with proliferation, invasion and risk for metastasis. It is expressed in various carcinomas and lymphomas like colorectal, breast, lung, skin carcinomas and follicular lyomphoma.[4] In invasive carcinoma of breast NST, it is expressed in 75 percent of cases. [5]

Although classified as an anti-apoptotic factor, it is linked to a more favourable prognosis and enhanced survival.[6] This association is attributed to its correlation with positive prognostic indicators such as oestrogen receptor (ER) status, progesterone receptor (PR) status, HER2 expression, and Ki-67 proliferation index.[7] It serves not only as a prognostic factor but also as a predictive marker. Novel therapies targeting anti-apoptotic proteins such as BCL-2 are currently undergoing clinical trials.[8]

MATERIAL AND METHODS

In this cross- sectional study, 71 cases of trucut breast biopsies and modified radical mastectomy specimens, diagnosed with invasive breast carcinoma, no special type (NST), which were received in the Department of Pathology between April 2019 and April 2024 were included. Males and patients with lymph node metastasis were excluded from the study. After fixation and grossing of the specimen, sectioning and embedding was done in paraffin blocks followed by cutting, slide preparation, and staining of the tissue with haematoxylin and eosin. Histopathological diagnoses and Nottingham scoring was done. For immunohistochemical stain, four-micron thin sections of these blocks were made and was applied according

to laboratory protocol.

The antibodies used includes ER (PA0151, Leica Bond, IL, USA), PR (PA0321, Leica Bond, IL, USA), HER2 (PA0571, Leica Bond, IL, USA), Ki-67 (PA0230, Leica Bond, IL, USA), and BCL2 (BCL2/100/D5, Leica Bond, IL, USA).

Allred scoring system was used for reporting ER and PR, by utilizing the intensity of staining on a scale of 0–3 and proportion of tumour cells being stained on a scale of 0–5, for a possible total score of 8 on summation. For Ki-67 nuclear staining was considered positive and no cut-off for positivity was decided. HER2 overexpression was evaluated using scoring criteria established by the College of American Pathologists. BCL2 staining was considered to be positive for cytoplasmic staining >10 % of malignant cells. The cases were classified into molecular subtypes: luminal type A, luminal type B, HER2-enriched, and basal-like. Luminal type A was characterized by ER and/or PR positivity and HER2 negativity, with a low proliferative index (Ki-67 <14%). Luminal type B exhibits ER and/or PR positivity with variable HER2 expression and a higher proliferative index (Ki-67 > 14%). The HER2-enriched subtype shows negative expression of ER and PR while being strongly positive for HER2. The basal-like subtype, also known as triple-negative breast carcinoma, lacks expression of ER, PR, and HER2. Quantitative variable like age were calculated as mean. Qualitative variables like ER, PR, HER2, KI-67 and BCL2 expression was calculated as frequency and percentage. The chi-squared test and Fischer exact was used to assess associations between different variables, with statistical analysis conducted using SPSS software version 26 (IBM, Armonk, NY, USA), and a *p*-value of <0.05 was considered statistically significant.

RESULT

The study included a sum of 71 cases. Participants remained divided into two age groups that includes <40 years and ≥40 years with a mean age of 46 years. Minimum age was 17 years while maximum age limit was 74 years. Clinic-pathological correlation among different parameters have been discussed in table 1 below. When BCL-2 positivity was considered in correlation with ER and PR positive patients it was statistically significant with *p*-values of 0.004 and 0.001 respectively according to Fischer exact test as shown in table 2 and 3 correspondingly.

Conversely, correlation of BCL 2 expression with HER2 was when analyzed this association was not statistically significant by applying Fischer exact test (*p*-value = 0.39) as shown in table 4.

When the expression of BCL2 was analyzed in terms of molecular subtypes it was found statistically insignificant (Pearson Chi-Squar 0.006) as shown in table 5.

Correlation of BCL2 expression with tumour grades and ki-67 proliferation index is shown in table 6 and 7 respectively, found statistically insignificant.

Table-1: Clinic-pathological correlation among different parameters

Clinic-pathological parameters		N (%)
age	<40	20 (28.17%)
	≥40	51 (78.83%)
grade	1	10 (14.08%)
	2	48 (67.61%)
	3	13 (18.31%)
Ki-67	<14	8 (11.27%)
	>14	63 (88.73%)
ER	Negative	33 (46.48%)
	positive	38 (53.52%)
PR	Negative	42 (59.15%)
	positive	29 (40.85%)
Her2	negative	46 (64.79%)
	positive	25 (35.21%)
Molecular subtype	Luminal A	6 (8.45%)
	Luminal B	34 (47.89%)
	Her2 enriched	18 (25.35%)
	Basal like	13 (18.31%)
BCL2	negative	28 (39.44%)
	positive	43 (60.56%)

Table-2: Correlation of BCL 2 expression with Oestrogen receptors positive cases

		BCl2		Total
		N	P	
ER	N	19	14	33
	P	9	29	38
Total		28	43	71

Table-3: Correlation of BCL 2 expression with Progesterone receptors positive cases

		BCl2		Total
		N	P	
PR	N	23	19	42
	P	5	24	29
Total		28	43	71

Table-4: Correlation of BCL 2 expression with HER2 positive cases

		BCl2		Total
		N	P	
HER2	N	10	18	28
	P	18	25	43
Total		28	43	71

Table-5: Correlation of BCL 2 expression with different molecular subtypes

		BCl2		Total
		N	P	
Molecular subtype	**luminal A**	3	3	6
	luminal B	8	26	34
	HER2 enriched	13	5	18
	Basal like	4	9	13
Total		28	43	71

Table-6: Correlation of BCL2 expression with tumour grades Pearson chi-square 0.350 (statistically insignificant)

		BCl2		Total
		N	P	
Grade	1	6	4	10
	2	17	31	48
	3	5	8	13
Total		28	43	71

Table-7: Correlation of BCL2 expression with ki-67 proliferation index Fischer exact test p 0.249 (statically insignificant)

		BCl2		Total
		N	P	
ki_67	<14%	5	3	8
	>14%	23	40	63
Total		28	43	71

DISCUSSION

The mean age was 46 years, with 20% of females being under 40 years and 72% being 40 years or older. This distribution is comparable to that observed in the Iranian female population with breast carcinoma, where approximately 47 years is an average age.[9] We observed highest frequency of breast carcinoma in individuals over 40 years of age, consistent with findings from Kang et al., who reported a peak incidence in the 40 to 49-year age group.[10] Similarly, Salvatorelli *et al*. reported that 40% of breast carcinoma cases occurred in the 40 to 49-year age group.[11] We observed high proliferation rate in 88 percent of cases which is similar to the value of 87 percent found by Ahmed *et al*.[12.]

Luminal cases were more prevalent in present study compared to non-Luminal cases, which is consistent with findings reported by Acheampong *et al.* [13]

Similarly, Łukasiewicz *et al*. found that 70% of cases were classified as Luminal A. In contrast, our study observed that among Luminal cases, a smaller proportion were classified as Luminal A, while the majority were Luminal B. This finding is contrary to the results reported by Pandit *et al*.[15]

In our study, 61% of cases exhibited BCL-2 positivity, compared to 66% reported by Pandey *et al.,* who found BCL-2 positivity in 72% of Luminal A and B cases, 46% of triple-negative cases, and 62% of HER2-enriched cases. In our study, BCL-2 positivity was observed in 50% (3 of 6) cases of Luminal A carcinomas, 70% (26 of 34) cases of Luminal B type carcinomas, 69% (9 of 13) cases of Basal-like carcinomas, and 28% (5 of 18) cases of HER2-enriched carcinomas. BCL-2 expression was significantly associated with the expression of hormone receptors including ER and PR ($p = 0.00$).[16]

In our study, BCL-2 positivity was observed in 40% of Oestrogen receptor positive cases and 33% of Progesterone receptors positive cases, whereas Sharmila et al. reported BCL-2 positivity in 58% of both ER-positive and PR-positive cases. For HER2-positive cases, BCL-2 positivity was found in 35% of cases in our study, a result that was not statistically significant, consistent with Sharmila et al.'s findings. Additionally, Sharmila et al. noted a decrease in BCL-2 positivity with increasing tumour grade, reporting BCL-2 positivity in 45.5% of Grade 1 invasive breast carcinoma NOS cases and 14.3% of Grade 2 invasive breast carcinoma cases. In contrast, our study found an increase in BCL-2 expression with higher tumour grades.[17]

CONCLUSION

These outcomes specify that BCL-2 positivity is related with the expression of hormone receptors, which are generally considered favorable prognostic factors, as supported by previous studies. However, no substantial relationship was amid between BCL-2 expression and Ki-67, HER2 status, or tumour grade. These factors are typically associated with poorer outcomes and thus did not demonstrate a significant association with BCL-2 expression.

AUTHORS' CONTRIBUTION

NN, SJ, NB: Concept, literature search, write-up, proof reading. FAF, SNK, SK: Data collection, data analysis, data interpretation.

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