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The Study on BCL2 Expression in Breast Carcinoma: Correlation with Predictive and Prognostic Factors

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Abstract: ***Breast cancer is a significant cause of mortality in Indian women, characterized by increasing incidence in younger individuals and heightened aggressiveness [1, 2]. The understanding of molecular subtypes, especially those defined by hormone receptor expression (ER, PR, HER2), is pivotal for clinical management [3, 4]. BCL2, a proto-oncogene, adds complexity as its overexpression in breast cancer paradoxically associates with improved survival [6]. This study aims to investigate BCL2 expression in breast carcinoma and its relationship with other prognostic markers. A total of 83 breast cancer cases were analyzed, with immunohistochemistry conducted for ER, PR, HER2, and BCL2. BCL2 cytoplasmic expression in invasive breast carcinoma was correlated with established prognostic factors, including tumor size, histological grade, lymph node status, hormone receptor status (ER, PR), HER2 expression, and Nottingham prognostic index. Results revealed that 81.9% of participants were aged over 50, and 81.9% exhibited BCL2 positivity out of which 52.9% of the cases had grade 3 BCL2 staining intensity, 35.3% showed grade 2 positivity, and only 11.8% had grade 1 BCL2 staining intensity. Notably, BCL2 expression significantly correlated with ER and PR status ($p < 0.05$). In conclusion, this study highlights the intricate role of BCL2 in breast cancer and its connection with critical prognostic factors. The significant association of BCL2 with ER and PR positivity underscores its potential as both a prognostic and therapeutic marker in breast cancer [3].***

Keywords: Breast Cancer, BCL2 Expression, Molecular Subtypes, Hormone Receptor Status

1. Introduction

Breast cancer presents a significant global health challenge, particularly in India where a trend of increasing incidence among younger women and greater aggressiveness has emerged. Sociocultural factors such as reduced breastfeeding, delayed childbirth, and limited healthcare access contribute to this complexity.

In the field of breast cancer research, molecular classification has gained prominence, guiding clinical management based on biomarker expression, including the estrogen receptor (ER), progesterone receptor (PR), and Human Epidermal Growth Factor receptor 2 (HER2). This classification divides breast cancers into ER Positive (HER2 negative), HER2 Positive (ER-positive or negative), and triple-negative (ER, PR, HER2 negative) groups, impacting treatment responses and patient outcomes.

Amidst this intricate landscape, the proto-oncogene BCL2 (B cell lymphoma 2) has become a focus of interest. Originally associated with adverse outcomes in certain non-Hodgkin lymphomas, BCL2 protein overexpression has been observed in various solid organ malignancies, including breast cancer. This paradoxical role of BCL2, acting as both a pro-tumorigenic anti-apoptotic factor and an inhibitor of proliferation associated with improved survival, prompts intriguing questions.

This study investigated the complexity of BCL2 expression across different histological types of breast carcinoma. This study aimed to unravel the relationship between BCL2 expression and established prognostic and predictive factors, including tumor size, histological grade, lymph node status, hormone receptor status (ER, PR), HER2 expression, and the Nottingham prognostic index. By employing gene expression profiling techniques, we rigorously analyzed the data to elucidate BCL2's multifaceted role in breast carcinoma.

Although potential biases and limitations are acknowledged, our research seeks to contribute to the growing understanding of BCL2 in breast cancer. In doing so, we hope to refine clinical management strategies and provide valuable insights into breast cancer prognosis and therapy.

2. Materials and Methods

This cross-sectional study was conducted at the Department of Pathology, SreeGokulam Medical College and Research Foundation from January 2022 to February 2023. The study population comprised clinically diagnosed female breast carcinoma patients who had undergone surgical intervention at SGMC and RF. The inclusion criteria included female breast carcinoma cases with surgical intervention, while the exclusion criteria included male breast carcinoma cases, those without available tumor blocks, and individuals who had received prior radiotherapy or chemotherapy. A sample size of 83 was determined based on the study by Eom et al.

Consecutive sampling of H&E and IHC slides was performed until the sample size was achieved. Statistical analysis was performed using SPSS Version 27 with a significance threshold of P <0.05. Ethical approval was obtained from the institutional committee.

3. Methodology

A total of 83 breast carcinoma cases, including special subtypes, were subjected to histopathological examinations. Hematoxylin and Eosin (H&E) slides were meticulously reviewed to establish the diagnosis and determine tumor subtypes. The specimens were processed, embedded in paraffin, and sectioned. Hematoxylin and eosin (H&E) staining was performed to facilitate histopathological examination. Immunohistochemistry (IHC) was performed specifically for BCL2, with BCL2 positivity defined as the presence of more than 10% cytoplasmic staining in invasive breast carcinoma. Additionally, the intensity of BCL2 expression was scored (score 0 – for no staining, score 1 – slight staining in some or most of the cells, score 2 – moderately strong staining, score 3 – strong staining in almost all the cells) along with immunoreactivity for the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), which was meticulously assessed and compared with BCL2 expression.

4. Results

Table 1: Age distribution of patients

Age Category (years)	Frequency	Percentage (%)
<50 years	15	18.1
≥ 50 years	68	81.9
Total	83	100

The mean age of the patients was 59.42±11.15 years. The minimum age was 34 years and the maximum age was 91 years. Majority (68%) of the patients belonged to ≥50 year age group.

Table 2: BCL2 expression and age

Age (years)	BCL2		χ^2	p value
	Positive (n=68) n (%)	Negative (n=15) n (%)		
<50 years	13 (19.1)	2 (13.3)	0.28	0.73
≥ 50 years	55 (80.9)	13 (86.7)		

19.1% of patients in the positive BCL2 group were in the aged group of <50 years when compared to those in the negative BCL2 group (13.3%). There was no statistically significant association between BCL2 expression and age (p >0.05).

Menopausal status of patients

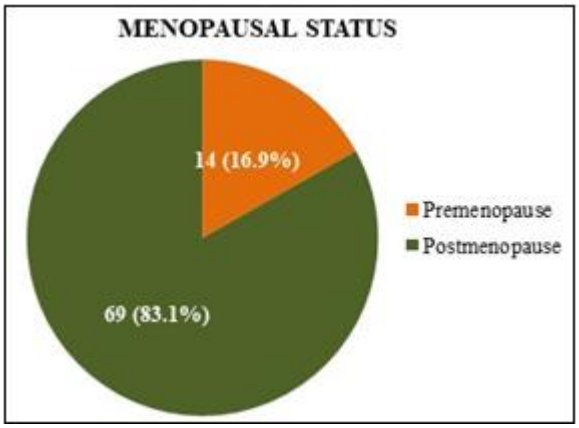

Figure 1

Table 3: BCL2 expression and menopausal status

Menopausal Status	BCL2		χ^2	p value
	Positive (n=68) n (%)	Negative (n=15) n (%)		
Premenopause	12 (17.6)	2 (13.3)	0.16	1.00
Postmenopause	56 (82.4)	13 (86.7)		

17.6% of patients in positive BCL2 group were in the premenopausal category when compared to those in negative BCL2 group. However, this difference was not statistically significant (p >0.05).

Table 4: Distribution of patients based on tumour size

Tumour Size	Frequency	Percentage (%)
<2 cm	15	22.1
2-50 cm	51	75.0
>5cm	2	2.9
Total	68	100

Table 5: BCL2 expression and tumour size

Tumour Size	BCL2		χ^2	p value
	Positive (n=56) n (%)	Negative (n=12) n (%)		
<2 cm	12 (21.4)	3 (25.0)	2.13	0.32
2-50 cm	43 (76..8)	8 (66.7)		
>5cm	1 (1.8)	1 (8.3)		

More than three forth (76.8%) of the patients in the positive BCL2 group had breast tumor size of 2-5cm, which was higher than that in the negative BCL2 group (66.7%). However, there was no statistically significant association between tumour size and BCL2 expression (p >0.05).

Table 6: BCL2 expression and lymph node status

Lymph Node Status	BCL2		χ^2	p value
	Positive (n=56) n (%)	Negative (n=12) n (%)		
N0	40 (71.4)	8 (66.7)	2.02	0.77
N1	11 (19.6)	3 (25.0)		
N2	2 (3.6)	1 (8..3)		
N3	1 (1.8)	0 (0.0)		
Not Assessed	2 (3.6)	0 (0.0)		

Majority of patients in both BCL2 groups (positive, 71.4%; negative, 66.7%) had N0 as the lymph node status. There was no statistically significant association between lymph node status and BCL2 expression (p >0.05).

Table 7: BCL2 expression and histological variants of breast cancer

Histological Variants	BCL2		χ^2	p value
	Positive (n=68) n (%)	Negative (n=15) n (%)		
IDC- NOS	49 (60.3)	13 (66.7)	9.64	0.31
ILC	8 (11.8)	0 (0.0)		
Metaplastic	2 (2.9)	0 (0.0)		
Mucinous	5 (7.4)	0 (0.0)		
Encapsulated papillary carcinoma	1 (1.5)	1 (6.7)		
Micropapillary	2 (2.9)	0 (0.0)		
Tubular	1 (1.5)	0 (0.0)		
Neuroendocrine	1 (1.5)	0 (0.0)		
Pleomorphic Lobular	0 (0.0)	1 (6.7)		

Among the histological variants of breast cancer assessed, patients in the positive BCL2 group with IDC-NOS (60.3%),ILC (11.8%), metaplastic (2.9%), mucinous (7.4%), micropapillary (2.9%), tubular (1.5%), and neuroendocrine (1.5%) variants was more when compared to that in the negative BCL2 group. However, there was no statistically significant association between histological variants of breast cancer and BCL2 expression (p >0.05).

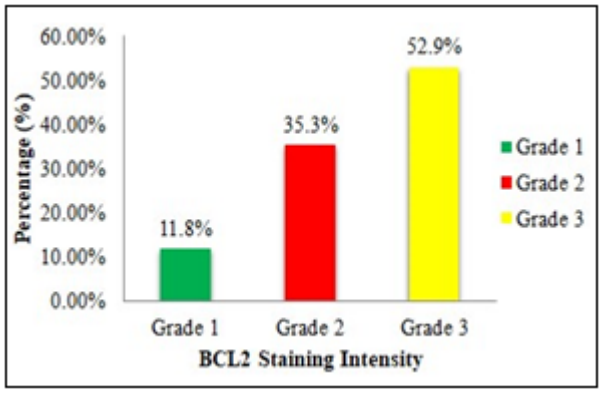

Figure 2: Distribution of study participants based on BCL2 staining intensity

More than half (52.9%) of the patients had grade 3 BCL2 staining intensity and only 11.8% had grade 1 BCL2 staining intensity.

Table 8: BCL2 expression and NPI grade of breast cancer

NPI grade	BCL2		χ^2	p value
	Positive (n=68) n (%)	Negative (n=15) n (%)		
Grade 1	17 (25.0)	1 (6.7)	2.46	0.30
Grade 2	38 (55.9)	11 (73.3)		
Grade 3	13 (19.1)	3 (20.0)		

Quarter of the patients in the positive BCL2 category had NPI grade 1 when compared to those in the negative BCL2 category (6.7%), and this difference observed was not found to be statistically significant (p value >0.05).

Table 9: BCL2 expression and TNM stage

TNM stage	BCL2		χ^2	p value
	Positive (n=56) n (%)	Negative (n=12) n (%)		
1A	11 (19.6)	3 (25.0)	4.81	0.36
2A	33 (58.9)	5 (41.7)		
2B	8 (14.3)	2 (16.7)		
3A	1 (1.8)	1 (8.3)		
3C	2 (3.6)	0 (0.0)		
Not Assessed	1 (1.8)	1 (8.3)		

Majority (58.9%) of the patients in positive BCL2 category had TNM stage of breast cancer as 2A which is higher than that in the negative BCL2 category (41.7%). No statistically significant association was observed between the TNM stage and BCL2 expression in breast cancer (p >0.05).

Table 10: BCL2 expression with ER, PR and HER2 status

Variables	BCL2		χ^2	p value
	Positive (n=45) n (%)	Negative (n=12) n (%)		
ER				
Positive	40 (88.9)	2 (16.7)	25.48	<0.001
Negative	5 (11.1)	10 (83.3)		
PR				
Positive	31 (68.9)	4 (33.3)	5.05	0.04
Negative	14 (31.1)	8 (66.7)		
HER2				
Positive	7 (15.6)	5 (41.7)	3.89	0.10
Negative	38 (84.4)	7 (58.3)		
ER, PR and HER2 Negative				
Yes	1 (2.2)	5 (41.7)	15.65	0.001
No	44 (97.8)	7 (58.3)		

Majority of the patients in positive BCL2 group had positive ER (88.9%), PR (68.9%) when compared to those in the counterpart group, difference observed was found to be statistically significant.

Triple-negative cases were found to be statistically significant when compared with their counterparts (p <0.05).

Table 11: BCL2 expression and local recurrence

Local Recurrence	BCL2		χ^2	p value
	Positive (n=28) n (%)	Negative (n=7) n (%)		
Yes	1 (3.6)	1 (14.3)	1.12	0.36
No	27 (96.4)	6 (85.7)		

Approximately3.6% of the patients in positive BCL2 group had history of local recurrence, which was lower than that in the negative BCL2 group (14.3%), and this difference observed was not found to be statistically significant (p value >0.05).

Table 12: BCL2 expression and distant metastasis

Distant Metastasis	BCL2		χ^2	p value
	Positive (n=30) n (%)	Negative (n=8) n (%)		
Present	0 (0.0)	1 (12.5)	3.85	0.21
Absent	30 (100.0)	7 (87.5)		

All the patients in the positive BCL2 group had no distant metastasis, which was higher than that in the negative BCL2

group (87.5%), and this difference observed was not found to be statistically significant (p value >0.05).

Table 13: BCL2 expression and vital status

Vital Status	BCL2		χ^2	p value
	Positive (n=30) n (%)	Negative (n=8) n (%)		
Alive	30 (100.0)	7 (87.5)	3.85	0.21
Dead	0 (0.0)	1 (12.5)		

All the patients in the positive BCL2 category were alive which was higher than those in the negative BCL2 category (87.5%). There was no statistically significant association between the vital status and BCL2 expression (p >0.05).

Images

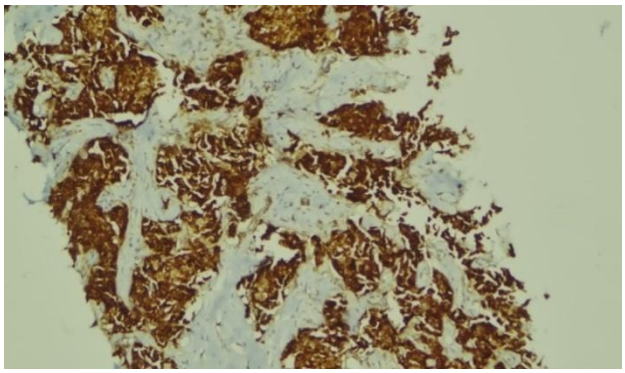

Figure 3: BCL2 showing strong (grade 3) staining

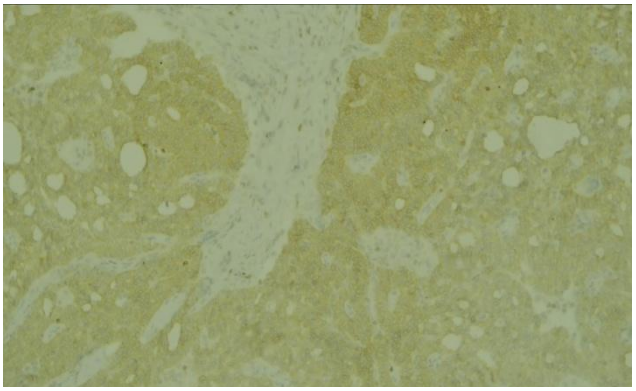

Figure 4: BCL2 showing moderate (grade 2) staining

Figure 5: BCL2 showing mild (grade 1) staining

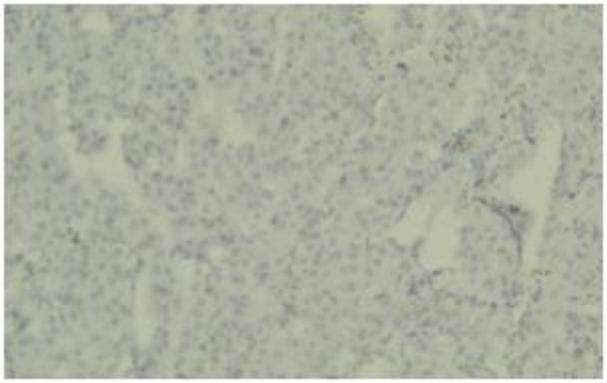

Figure 6: BCL2 negativity

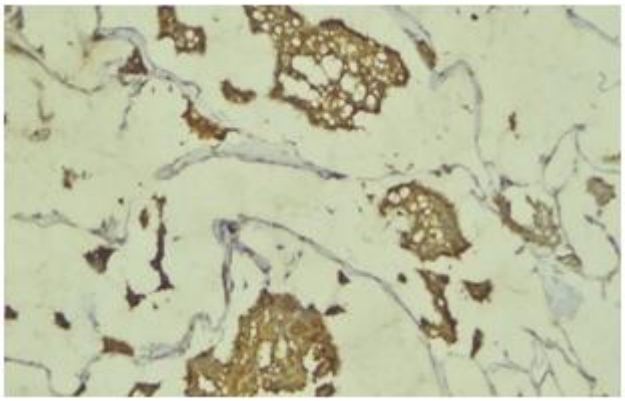

Figure 7: BCL2 positivity inmucinous carcinoma

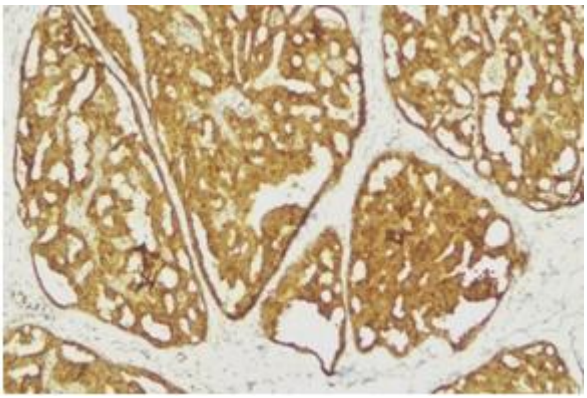

Figure 8: BCL2 positivity in IDC-NOS(2)

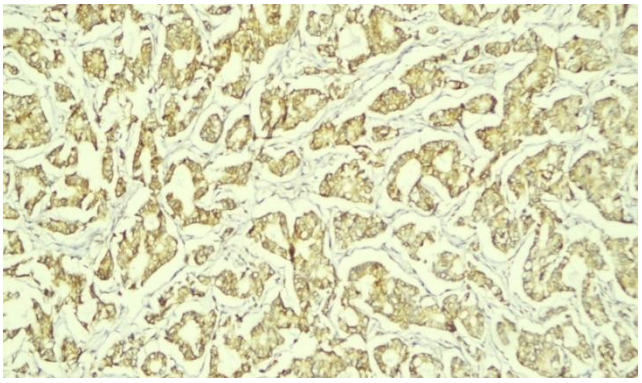

Figure 9: BCL2 positivity intubular carcinoma

5. Discussion

The present study revealed that BCL2 expression was detected in 81.9% of the cases, with varying staining intensities. Grade 3 BCL2 staining intensity was the most

prevalent (52.9%), followed by grade 2 (35.3%) and grade 1 (11.8%). These findings are consistent with prior research by P. Helleman et al., who reported 75% BCL2-positive cases in their study of 251 breast carcinoma cases. Regarding the age distribution, our study noted a higher prevalence of BCL2 expression in patients over 50 years of age, although statistical significance was not reached (p=0.383). In contrast to our findings, Johnson et al. and Eom et al. observed lower BCL2 expression levels in younger breast cancer patients (<40 years).

In terms of menopausal status, our study indicated higher BCL2 positivity in postmenopausal patients, although statistical significance was not observed (p=1.00). Conversely, Yu et al. found a statistically significant association between BCL2 expression and menopausal status. The examination of factors such as tumor size, Nottingham Prognostic Index (NPI) grading, histological subtype, lymph node status, and TNM stage in relation to BCL2 expression did not yield statistically significant correlations, consistent with some previous studies, but contradicting others. Notably, our study found a strong association between BCL2 expression and estrogen receptor (ER) and progesterone receptor (PR) positivity (p=0.001 and p=0.04, respectively), in line with previous research indicating that BCL2 expression is linked to ER and PR positivity, both of which are favourable prognostic markers. Furthermore, our study revealed that triple-negative breast cancer (TNBC) cases exhibited lower BCL2 positivity, with a statistically significant difference compared to non-TNBC cases (p<0.001), which is consistent with other studies highlighting the inverse correlation between BCL2 expression and TNBC. While our study did not establish significant correlations between BCL2 expression and local recurrence, distant metastasis, or vital status, Eom et al. demonstrated that BCL2-positive expression was associated with favourable 5-year relapse-free survival and disease-specific survival in patients with breast cancer.

6. Summary & Conclusion

In this study, involving 83 cases of invasive breast carcinoma, BCL2 expression was detected in the majority of patients (81.9%), with grade 3 staining intensity being the most prevalent (52.9%). The patient cohort was diverse in age, with the majority being over 50 years old, and invasive ductal carcinoma (IDC-NOS) was the most common histological type. Notably, all mucinous carcinoma cases exhibited strong BCL2 expression. Although BCL2 expression was not significantly associated with the Nottingham Prognostic Index (NPI), it demonstrated a strong and statistically significant correlation with estrogen receptor (ER) and progesterone receptor (PR) positivity, highlighting its link with favourable hormonal receptor status. Furthermore, BCL2 was absent in triple-negative breast cancer cases, indicating a significant inverse correlation. However, BCL2 expression was not significantly associated with tumor grade, lymph node status, local recurrence, distant metastasis, or vital status in this cohort of patients with breast carcinoma.

In summary, this study highlights the significance of BCL2 expression in invasive breast ductal carcinoma. BCL2 expression was directly related to ER and PR positivity, whereas it was inversely related to triple-negative cases. These findings suggest potential avenues for enhancing the efficacy of endocrine treatment of ER+ breast cancer by targeting the antiapoptotic effect of BCL2 using BH3 mimetics. Moreover, BCL2 antagonism in ER+ tumors may enhance the effectiveness of pro-apoptotic drugs in comparison to conventional treatments.

Broader Implications

The findings of this study have broad implications for breast cancer treatment and personalized medicine. Understanding the role of BCL2 expression in different breast cancer subtypes can guide treatment decisions. Targeting BCL2 using BH3 mimetics may open new therapeutic avenues for ER+ breast cancer patients who often have limited treatment options. However, further research with larger sample sizes and a broader spectrum of cases is necessary to validate these findings and develop effective targeted therapies. This study contributes to the growing body of knowledge aimed at improving breast cancer treatment outcomes and underscores the importance of personalized approaches in cancer therapy.

Ethical Compliance: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional committee.

Conflict of Interest declaration: The authors declare that they have NO affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

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