



Original Research Article

BCL2 expression in ductal carcinoma of breast and its association with other clinicopathologic variables

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ABSTRACT

Breast cancer has now become the most common cancer among Indian females exceeding cervical cancer. Age adjusted incidence rate as high as 25.8 per 100,000 women and mortality 12.7 per 100,000 women. Trends of breast cancer now in India are increasing incidence at younger age group, late stage of presentation, aggressive cancers in the young. Bcl 2-a protooncogene identified in 1984. The protein is found mainly in the periphery of mitochondria, on the perinuclear membrane, and in the endoplasmic reticulum. In our study bcl2 expression in different histologic types of breast carcinoma and correlates we read about its expression with other clinico pathologic variables such as tumour size, histological grade, lymph node status, hormone receptor status and Nottingham prognostic index. In this study 23.3% (7 out of 30) of the cases showed positivity for bcl2 expression of which all cases were strongly positive. Bcl2 expression was found in 45.5% grade I well differentiated ductal carcinoma, 14.3% of Grade II Moderately differentiated ductal carcinomas, all cases of mucinous carcinoma of breast ($p < 0.025$) Bcl2 and ER/PR status found to have a statistically significant direct correlation with p value < 0.004 . Hence Bcl2 antagonism in these ER+ tumors might increase the effectiveness of these predominately proapoptotic treatment even more than it does from combination with endocrine therapies. Further development of bcl2 inhibitors has been explored and small molecule inhibitors such as ABT-737 AND ABT-199 have been recently introduced. Emerging evidence also suggests the usefulness of this type of therapy in breast cancer.

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1. Introduction

Breast cancer has now become the most common cancer among Indian females exceeding cervical cancer. Age adjusted incidence rate as high as 25.8 per 100,000 women and mortality 12.7 per 100,000 women. Trends of breast cancer now in India are increasing incidence at younger age group, late stage of presentation, aggressive cancers in the young. .

Almost all breast malignancies are adenocarcinomas (>95%). In the most clinically useful classification system, breast cancers are divided based on the expression of hormone receptors-estrogen receptor (ER) and progesterone receptor (PR) and the expression of human epidermal

growth factor receptor 2 (HER2, also known as ERBB2), into three major groups:

1. ER positive (HER2 negative; 50-65% of cancers)
2. HER2 positive (ER positive or negative; 10-20% of cancers)
3. Triple negative (ER, PR, HER2 negative; 10-20% of cancers)

These three groups show striking differences in patient characteristics, pathologic features, treatment response, metastatic patterns, time to relapse and outcome. Within each group are histologic subtypes, some of which also have clinical importance. The prognosis and treatment of breast carcinoma depends on tumour size, histological grade, lymphnode stage, expression of estrogen receptor,

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progesterone receptor, over expression of her2/neu. Gene expression profiling is becoming standard care for breast cancer patients, but is available in selected institutions and is subject to issues like feasibility, interpretation and cost. These are of critical relevance in considering the need to identify molecular features of individual tumours in routine practice.

This includes ER, PR, HER2, P53, KI67. In addition markers of angiogenesis and apoptosis are used. Identifying prognostic molecular markers recently has become the objective in translational research studies on breast carcinoma. These markers potentially could serve as a complement to clinico pathologic staging in identifying patients who have high risk for disease recurrence and who need systemic therapy.¹Bcl 2-a protooncogene identified in 1984. The protein is found mainly in the periphery of mitochondria, on the perinuclear membrane, and in the endoplasmic reticulum.

About 70% of cases of follicular lymphoma and 20% of cases of diffuse B-cell lymphomas have increased concentrations of Bcl-2 protein as a result of the t(14;18)(q32;q21) translocation which positions the Bcl-2 gene under the control of the strong Ig heavy-chain promoter gene. The fused Bcl-2-Ig gene generates chimeric mRNAs that consist of Bcl-2 at 5' portion and immunoglobulin at 3' portion, and the chimeric mRNA contains the Bcl -2 coding frame for a 239-amino acid polypeptide. In consequence, high levels of Bcl-2 protein are generated. However, a discrepancy in the relationship between the occurrence of t(14;18) translocation, Bcl-2 gene rearrangement, and over expression of Bcl-2 protein has been found in lymphoma, which suggests the existence of several molecular mechanisms for Bcl-2 protein over expression. A number of investigators have shown that bcl2 is a candidate prognostic marker. In our study bcl2 expression in different histologic types of breast carcinoma and correlates we read about its expression with other clinico pathologic variables such as tumour size, histological grade, lymph node status, hormone receptor status and Nottingham prognostic index

2. Aim of the study

To assess Bcl2 expression in breast carcinoma and its correlation with other clinicopathologic factors such as tumour size, lymph node status, histological grade, ER, PR and HER2/ neu expression and Nottingham prognostic index.

2.1. Objectives

1. To analyze Bcl2 expression in invasive ductal carcinoma of breast
2. To correlate Bcl2 expression with ER, PR and HER2 neu expression.

3. To analyze the correlation of histological grade, Nottingham prognostic index of each case with Bcl2 expression.

3. Observation and Results

Table 1: AGE incidence of breast carcinoma

Age	Number of cases	Percentage (%)
<40 years	8	26.7
40-50 years	5	16.7
50-60 years	10	33.3
>60 years	7	23.3
Total	30	100.0

Table 2: BCL2 expression in breast carcinoma

BCL2 Exp	Number of cases	Percentage (%)
Negative	23	76.7
Positive	7	23.3
Total	30	100.0

Out of 30 cases we have taken, 7 cases(23.3%) shows intense grade(+++) III positivity, 23 cases (76.7%) shows no expression. Staining for Bcl-2 classified is into four groups:

1. No staining present in any of the breast cancer cells (-),
 2. Slight staining in some cells or in most of the cells (+),
 3. Moderately strong staining(++), or
 4. Strong staining present in almost all cells (+++).
- Classification was done by a senior pathologist.

Table 3: BCL2 expression with histopathological grade

Grade	BCL2 Expression		P value
	Positive	Negative	
I	5(45.5%)	6(54.5%)	.075
II	2(14.3%)	12(85.7%)	
III	0(0.0%)	5(100.0%)	

Bcl2 expression is seen in 45.5% cases of Grade 1 tumours, 14.3% cases of Grade2 tumours. All the high grade, Histologic Grade3 tumours shows no immunoreactivity for bcl2 expression.

Table 4: Correlation of BCL2 expression with ER status

ER	BCL2 Expression		P value
	Negative	Positive	
Negative	18(100.5%)	0(0.0%)	.000*
Positive	5(41.7%)	7(58.3%)	

*-statistically significant (P<0.05)

7 out of 12 (58.3%) ER+ Cases, shows positivity for bcl2 expression. This association was found to be statistically significant(p value<0.05)

Table 5: Correlation of BCL2 expression with PR status

ER/PR	BCL2 Expression		P V alue
	Negative	Positive	
Negative	18(100.0%)	0(0.0%)	.000*
Positive	5(41.7%)	7(58.3%)	

*-statistically significant (P<0.05)

7 out of 12 cases (58.3%) of PR+ breast carcinoma shows positivity for bcl2 expression. This association is found to be statistically significant with p value<0.05

Table 6: Association of BCL2 expression with Her2/Neu

HER2/NEU	BCL2 Expression		P value
	Negative	Positive	
Negative	19(73.1%)	7(26.9%)	.236
Positive	4(100.0%)	0(0.0%)	

Out of 30 cases,4 cases show positivity for Her2/neu expression. All those cases shows no immunoreactivity for Bcl2. Hence Bcl2 shows inverse relationship with Her2/neu.

Table 7: Correlation of BCL2 expression with tumour size

Tumour size	Bcl2 expression		P Value
	Negative	Positive	
T1	2(50.0%)	2(50.0%)	.397
T2	12(80.0%)	3(20.0%)	
T3	9(81.8%)	2(18.2%)	

Table 8: Correlation of BCL2 expression with lymphnode status

LYMPH node Status	BCL2 Expression		P Value
	Positive	Negative	
N0	3(33.3%)	6(66.7%)	.562
N1	2(25.0%)	6(75.0%)	
N2	2(25.0%)	6(75.0%)	
N3	5(100.0%)	0(0.0%)	

Table 9: Association of H R status with BCL2 expression with in ductal carcinoma nos type

HR	BCL2 Expression		P value
	Negative	Positive	
Negative	16(100.0%)	0(0.0%)	.004*
Positive	5(55.6%)	4(44.4%)	

*-statistically significant (P<0.05)

In ductal carcinoma NOS type, 44.4% of ER positive cases show Bcl2 positivity, which is statistically significant (p value-0.004)

3.1. Colour Plates

Fig. 1:

Table 10: Association of BCL2 expression with in other special histologic types of breast carcinoma

Special type	BCL2 expression		P value
	Negative	Positive	
Mucinous ca	0(0.0%)	3(100.0%)	.025*
Metaplastic & secretory cases	2(100.0%)	0(0.0%)	

*-statistically significant (P<0.05)

4. Discussion

4.1. Incidence

Invasive ductal carcinoma of breast is the third most frequent carcinoma reported in the Department of Pathology, Coimbatore Medical College and is accounting for 10.3% of total malignancies in the year 2017.

4.2. Age of occurrence

In the present study majority of invasive ductal carcinoma of breast cases belong to ages between 35 and 76 years. The mean age of invasive ductal carcinoma of breast in this study was 52 years. Hwang et al found 48.75 years as mean age of patients in their study. Abdel fatah et al in his study found that median age was 51 years.

4.3. Distribution of histological variants in our study

IDC-NOS type constitutes 83.3% of cases among the various histological variants included in the study.²Other special histological types (mucinous,metaplastic and secretory carcinoma) constitute the remaining part of study.

4.4. BCL2 expression in our study and comparative analysis

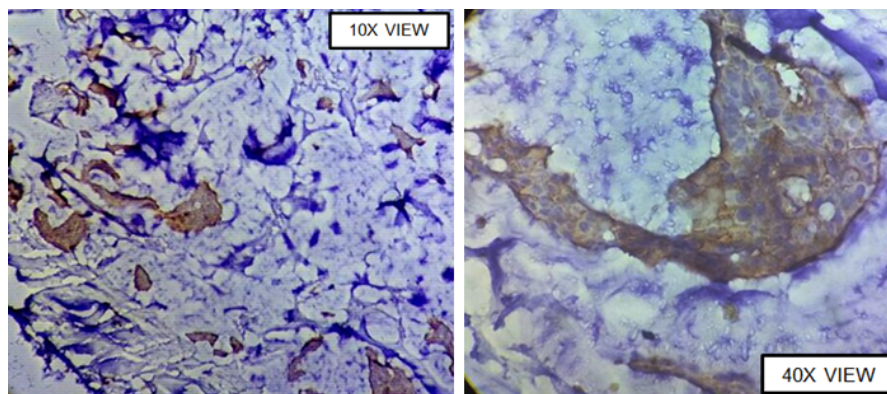
In this study 23.3% (7 out of 30) of the cases showed positivity for bcl2 expression of which all cases were strongly positive.

In a study done by Oakes et al 83%(45out of 54)of luminal tumours of breast showed Bcl2 expression.^{3,4}Hwang et al also found Bcl2 expression in 68.2%(4932/7230) of invasive ductal carcinoma of breast. We found that percentage of bcl2 positive cases decreases with increasing grade. About 45.5% cases of grade 1 Ductal ca NOS type and 14.3% of cases of grade 2 ductal carcinoma are positive for Bcl2 expression.

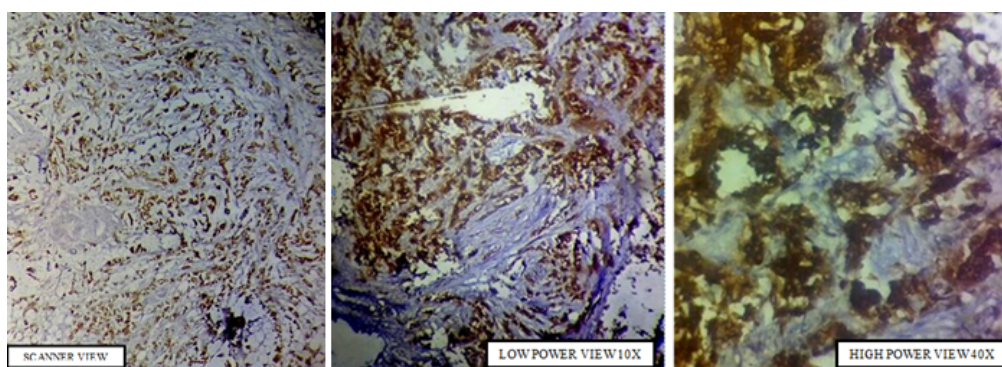
All mucinous carcinomas shows Bcl2 positivity with p value of p<0.025(statistically significant) and score 3+ was found in all the cases.Grade3 Poorly differentiated carcinomas shows no immunoreactivity with Bcl2. Joehnsu et al(1994) in his study found that Bcl-2 expression was particularly common in well differentiated carcinomas (83% moderately or strongly positive).

Table 11: Immunohistochemical marker expression with corresponding NPI groups

Marker	NPI1		NPI2		NPI3	
	+ VE	-VE	+ VE	-VE	+ VE	-VE
ER	7(70.0%)	3(30.0%)	1(16.7%)	5(83.3%)	4(28.6%)	10(71.4%)
PR	5(5.0%)	5(50.0%)	1(16.7%)	5(83.3%)	6(42.9%)	8(57.1%)
HER/Neu2	-	10(100.0%)	-	6(100.0%)	4(28.6%)	10(71.4%)
BCL Expression	5(5.0%)	5(50.0%)	1(16.7%)	5(83.3%)	1(7.1%)	13(92.9%)

**Fig. 1:** Immunohistochemistry of BCL2 in mucinous carcinoma of breast

(low power view showing clusters of cells with cytoplasmic expression of bcl2 seen in a background of mucin)
[high power view showing bcl2 expression.]

**Fig. 2:** BCL2 expression in ductal carcinoma of breast**Table 12:**

NPI	Value	Prognosis	15 year survival rate
1	<3.4	Good	80%
2	3.4-5.4	Moderate	42%
3	>5.4	Poor	13%

NPI=Tumour size in cm*0.2+histological grade(1-3)+number of positive lymphnodes (1=0nodes,2=1-3 nodes,3=>3 nodes)

4.5. BCL2 expression with other clinicopathologic variables:

Direct correlation between Bcl2 expression and tumour size is found in ductal carcinoma nos type with significant p value<0.024 that signifies smaller tumour shows more positivity for Bcl2 expression than larger size tumours.⁵For practical purpose according to AJCC TNM STAGING, tumours are segregated into T1-<2cm,T2-2-5cm,T3->5cm. Average tumour diameter was more than 2-5cm in patients with absence of Bcl2 expression.

Similarly according to AJCC TNM Staging, lymph node status were divided into N0-No nodal metastasis, N1-1-3 involved nodes,N2-4-9 nodes positive, N3-10 or more nodes shows positivity.^{6,7}Lymph node status doesn't appear to play a statistically significant role with Bcl2 expression.

5. Hormone receptor status with BCL2 expression

High levels of Bcl2 expression are strongly associated with positive expression of receptors for estrogen and progesterone. Bcl2+ evaluated cases show 100% positivity in relation to estrogen receptors and in 100% of cases positive reaction to progesterone receptors with statistically significant p value<0.04,these cases representing a good response rate to hormonal therapy.

Phenotype of Bcl2+/ER+/PR+(7 CASES) associated the lack of HER2 expression.⁸ Assessing the BCL2 expression according to molecular classification, we observed that luminal A type and normal breast like tumours expressed Bcl2 marker at a rate of 44.4 %, no immunoexpression noted in luminal B and remaining molecular subtypes.⁹

In breast cancer,Bcl2 expression is associated with markers of better differentiation(grade 1 lesions which are ER positive)as we confirmed with this work. Most previous studies of Bcl2 in breast cancer have also shown a favourable association between Bcl2 positivity and outcome at least in univariate analysis.⁸ In the breast, Bcl2 is expressed in normal glandular epithelium and is upregulated by estrogen possibly as a result of direct transcriptional induction with negative regulation by p53-dependant mechanisms.

In our study, we reported strong correlation between the presence of Bcl2 and estrogen receptor positivity; no relationship with lymphnode status, tumour size and inverse relationship with immunostaining for HER2.

5.1. BCL2 expression with nottingham prognostic index

The original Nottingham Prognostic Index is a numerical value which is calculated as follows.

Kurshumliu et al (author?)¹⁰ observed in their study, a strong correlation between ER and bcl-2 expression, and hence an inverse statistical correlation between bcl-2 and NPI value. This is also supported in the study by Zhang et al.¹¹ who concluded the following: 1) expression of bcl-2 is

associated with better response to hormonal therapy, and 2) expression of bcl-2 is a good prognostic marker irrespective of the nodal status. Some early studies such as one did by Zhang et al¹² have reported an inverse correlation between expression of bcl-2 and immunohistochemical detection of EGFR, Her-2/neu, and p53.

6. Conclusion

To conclude that,Bcl2 expression in invasive ductal carcinoma is directly correlated with lower histologic grade (well differentiated tumours), small tumour size, ER+, PR+ Status of individual, all cases of mucinous carcinoma of breast. It inversely correlated with Her2/neu status, other special histologic subtypes (metaplastic and secretory carcinoma).

These Encouraging data resulted here promises on a new approach, to enhance the efficacy of endocrine treatment in estrogen receptor-positive (ER+) breast cancer by negating the antiapoptotic effect of BCL-2 by using BH3 mimetics. Teixeira et al.(1995)¹¹ reported that MCF7 human ER+ breast cancer cells had increased sensitivity to the cytotoxic agent doxorubicin when treated with antisense BCL-2. Lastly, ER+ tumors found to have a reduced likelihood of complete pathological eradication with chemotherapy or anti HER2 therapies than ER-negative tumors. Hence Bcl2 antagonism in these ER+ tumors might increase the effectiveness of these predominately proapoptotic treatment even more than it does from combination with endocrine therapies

More recently, a number of BH3 mimetic small molecules, which mimic the action of proapoptotic BH3- only proteins, have been developed to counteract antiapoptotic proteins such as BCL-2 and BCL-2-related proteins BCL-XL and BCL-W. Further development of bcl2 inhibitors has been explored and small molecule inhibitors such as ABT-737 AND ABT-199 have been recently introduced. Emerging evidence also suggests the usefulness of this type of therapy in breast cancer. Hence Further studies are needed by involving larger scale of patients to assess Bcl2 expression across different molecular sub types of breast carcinoma and to enhance the effectiveness of new therapy targeting bcl2.

7. Source of funding

None.

8. Conflict of interest

None.

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