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Expression of Bcl2 and Ki67 in Breast Carcinoma and their Correlation with Histological Grade

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Abstract

Introduction: *In Breast Carcinoma classical biomarkers, namely, Estrogen receptor(ER), Progesterone receptor(PR), Her2neu and Ki67 possess established prognostic significance. Anti- apoptotic factor Bcl-2,anovel marker, is emerging as an important prognostic indicator. The present study focuses on correlation of newly emerging biomarker like Bcl-2 with Ki-67 score and histological grading.*

Materials and Methods: *A hospital based observational prospective study was conducted taking 52 mastectomy specimens. After gross examination, routine histopathology was done by Hematoxylin and Eosin stain (H&E). Immunohistochemistry was done for ER, PR, Her2, Ki-67 and Bcl-2. Cases were graded according to Nottingham Histologic score. Bcl-2 score was correlated with Ki67 scoring. One-Way ANOVA tests were done for association of histopathological grades with Bcl-2 and Ki67 score. Correlation between histopathological grade, Bcl-2 and Ki67 was performed using Pearson Correlation Coefficient.*

Results: *Histologically, 50 cases were invasive ductal carcinoma and 2 tubular carcinoma. 34(65%) cases were Grade 3. Higher Ki67 score were associated with higher grades (f ratio= 141.932, $p<0.0001$) and was positively correlated ($r=0.3468$, $p<0.041$). Bcl-2 score was inversely associated with Histological Grade (f ratio= 35.801, $p<0.001$).A strong negative correlation was also observed between Bcl-2 and Ki-67 expression ($r= -0.8896$, $p<0.001$).*

Conclusion*: Bcl-2 expression is associated with lower grade and less aggressive tumors. Bcl-2 is inversely related with Ki-67. Thus Ki-67 and Bcl-2 can be used to predict the aggressiveness of breast carcinoma.*

Keywords: *Breast carcinoma, Biomarkers, Histologic Grading, Bcl-2, Ki67*

Introduction

Breast carcinoma is one of the most common cancers in women throughout the world as well as in India. The estimated number of breast cancer cases in India during 2012 was 145,000 cases with age standardized incidence rate of 25.8 per 100,000 women [1,2]. Several studies have reported that breast carcinoma is often triggered by mutations that lead to an over-expression of biomarkers, namely, estrogen-receptor (ER), progesterone receptor (PR), Human Epidermal growth factor receptor 2 (HER2) and Ki-67 [3].

These markers have been demonstrated to be important prognostic factors for endocrine therapy [3,4]. Novel molecular markers, Bcl-2 are currently emerging as tools for prognostication of breast carcinoma, guiding therapy, and predicting treatment response. The present study will focus on correlation of newly emerging biomarkers like Bcl-2 with proliferation marker (Ki-67 score) and histological grading. Bcl-2 is apoptotic regulator having a role in maintaining the balance between cell proliferation and apoptosis. Hence its mutation can lead to cell immortalization and tumor progression5,6. On the other hand, Ki-67 is the proliferative index which is commonly utilized in molecular classification of breast cancers [7].

The Bcl-2 family protein, Bcl-2, is upregulated by estrogens in breast cancer, through a direct consequence of transcriptional induction. Some authors reported that Bcl-2 constitute a strong protein marker in breast cancer and is a favorable prognostic factor in ER positive breast cancer8. Recent studies have also suggested that Bcl-2 is a reliable prognostic marker, particular for hormone receptor (HR)-positive breast cancer [9]. Bcl-2-positive expression is associated with better outcomes of metastatic and early breast cancer treated with either hormone therapy or chemotherapy [10]. The mechanism for the differences in prognosis remains unclear, but Bcl-2 expression is dependent on the ER status, so the different roles of Bcl-2 appear to be dependent on the molecular subtype11. In this study, the role of Bcl-2 as a prognostic marker was investigated according to the 13th St. Gallen immunohistochemical classification.

Proliferative index, Ki-67 has a substantial role in molecular classification of breast cancers [12]. A cut-off Ki-67 score of 14% is used in classification of breast carcinoma at molecular level as Luminal A and Luminal B subtypes [13]. Although high Ki-67 expression was found to correlate with increased rates of clinical response and pathological complete response following neo-adjuvant chemotherapy (NAC) [14,15]. Its correlation with other biomarkers like Bcl-2 is necessary to evaluate the tumor grades and to determine their relation with different grades of breast carcinomas. Some studies have

shown that expression of tumor markers have a strong correlation with different tumor grades of breast cancer [16,17].

Only a few studies have been conducted worldwide to determine the correlation between molecular subtype, Ki-67 and recently emerging biomarker, Bcl-2. Moreover no such study has been done in this geographic area. Studies to determine the correlation between the histologic grading and biomarkers are sparse. The present study was conducted for establishing a correlation between ki67 and Bcl-2 in breast carcinoma and their relation with histologic grade.

Materials and Methods

The study was conducted in the Department of Pathology taking all mastectomy specimens received over a span of 12 months. It is a hospital based observational prospective study. Time frame of the study was January 2020 to December 2020.

All primary breast carcinoma patients who underwent mastectomy were included irrespective of their age. Patients who previously received chemotherapy/radiotherapy and those who refused to give consent for the study were excluded from the study. Data was collected from the histopathology request form sent along with the specimen to the Department of Pathology and also directly from the patient and their relatives. Gross examination of the specimens were done along with routine histological study by hematoxylin and eosin stain followed by immunohistochemistry for ER, PR, Her2, Ki-67 and Bcl- 2.

Primary Antibody (Immunostain Clone)

ER	Rabbit monoclonal antibody SP1
PR	Rabbit monoclonal antibody Y85
HER2	Rabbit monoclonal antibody SP3
Ki67	Rabbit monoclonal antibody SP6
Bcl2	Mouse monoclonal antibody 124

From routine H&E slides the breast carcinomas were classified into their respective histological types and they were graded according to Nottingham Modification of Scarf Bloom-Richardson Criteria (Table1).

Bcl-2 scoring was based on the percentage of Bcl-2 positive cells as was done in the study of Ayadi et al 18. Bcl-2 was graded according to the expression level ranging from low (% of positive cells ≤30%), moderate (>30% of positive cells ≤ 70%) to strong (% of positive cells > 70%).

The following scores were assigned as percentage of positively stained tumour cells.

Scoring of Ki-67 was based on the percentage of cells showing nuclear immunopositivity after reviewing the entire section selected for the immunostain.

Parameters	Frequency	Percentage (%)
SITE		
CENTRAL	9	17.3
LOWER INNER	6	11.5
LOWER OUTER	7	13.4
RETROAREOLAR	4	7.6
UPPER INNER	8	15.3
UPPER OUTER	18	34.6
FOCALITY		
MULTIFOCAL	7	13.4
UNIFOCAL	45	86.5
DCIS		
ABSENT	4	7.6
NOT IDENTIFIED	25	48.1
PRESENT	23	44.2
MARGIN		
INVOLVED	3	5.7
UNINVOLVED	45	86.5
LVI		
NOT IDENTIFIED	24	46.1
PRESENT	26	50.1
PNI		
NOT IDENTIFIED	27	51.9
PRESENT	18	34.6
HISTOLOGIC TYPE		
IDC, NST	51	98.1
TUBULAR	1	1.9
HISTOLOGIC GRADE		
GRADE-1	5	9.6
GRADE-2	13	25
GRADE-3	34	65.3
BIOMARKER		
ER	32	61.5
PR	16	30.7
HER2	4	7.6

Table 1: Distribution of the cases based on site, focality, presence/ absence of DCIS, status of margins, presence/absence of LVI and PNI, histologic type, histologic grade and biomarker status

Result analysis

Total 63 patients were initially evaluated for inclusion in the study. However, 7 patients had a history of previous chemotherapy and 4 patients underwent lumpectomy for which they were excluded from the study. Ultimately, 52 cases were selected for the present study.

The mean age of the patient population was (53.57 ± 10.5) years with an age range of 38 to 77 years. Only 2 cases in our study population were male breast carcinoma (TABLE 3). Distribution of histological types, histological grades and distribution of conventional biomarkers (ER, PR, Her2) are depicted in Tables 4 to 7 and Figures: 1 to 3.

An IHC score was generated as described in methods and quantification was performed of cytoplasmic expression for Bcl-2 and nuclear expression for Ki67. Bcl-2 has been graded according to the expression level ranging from low (% of positive cells ≤30%), moderate (>30% ≤ 70% of positive cells) to strong (% of positive cells > 70%).

In the present study, cases were sorted according to Nottingham grade, Ki-67 and Bcl-2 scoring depicted in Tables 8 to 10 and Figures 4 to 6. Distribution of Bcl-2 expression was maximum with Grade1 (67%) and distribution of Ki67 was maximum with Grade 3 (46%).

Statistical analysis was done using One-Way ANOVA tests to determine association of histopathological grades and different biomarkers (Bcl-2 and Ki- 67). Correlation between histopathological grade, Bcl-2 and Ki67 was performed using Pearson Correlation Coefficient Calculation.

BIOMARKER	GRADE1	GRADE2	GRADE3
Bcl-2	35 (67%)	9 (17%)	8 (16%)
Ki-67	5 (12%)	13 (31%)	24 (57%)

Table 2: Expression of Bcl-2 and Ki-67 with varying histologic grades: Ki-67 is positively correlated with histological grading ($r=0.3468$, $p<0.041$) and Bcl-2 was inversely correlated with histological grading ($r= -0.8983$, $p<0.001$).

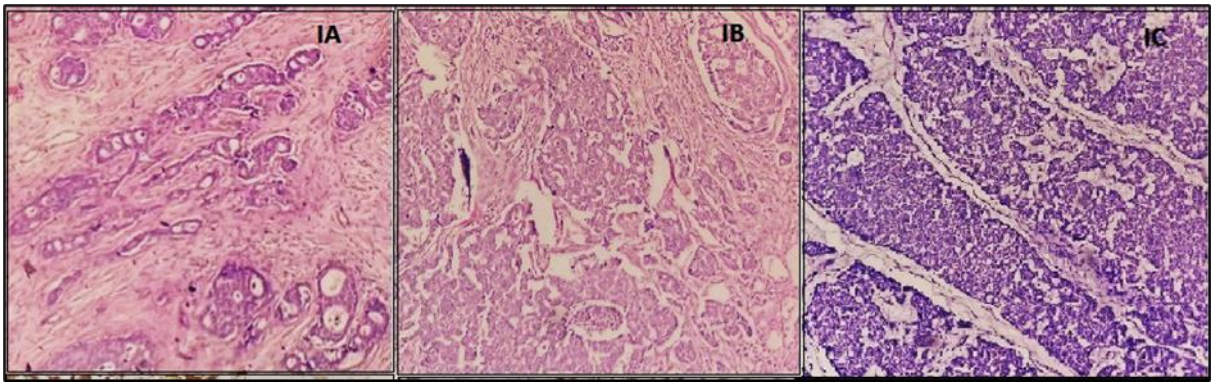

Figure 1: Different grades of invasive ductal carcinoma. Grade 1 (A), Grade 2 (B), Grade 3 (C). [H&E; 100X]

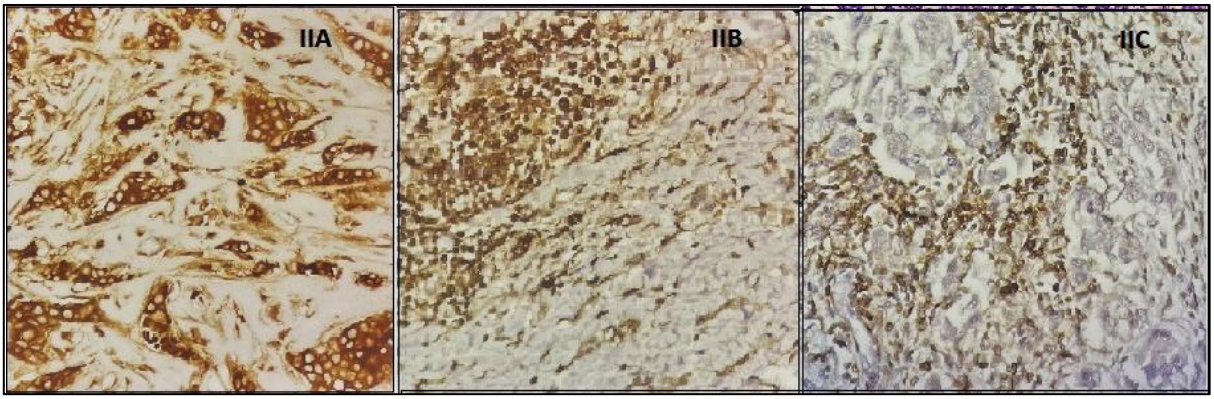

Figure 2: Corresponding immunohistochemistry with Bcl2 revealing progressively decreasing expression. Grade 1 (A), Grade 2 (B), Grade 3 (C). [DAB Chromogen; 400X]

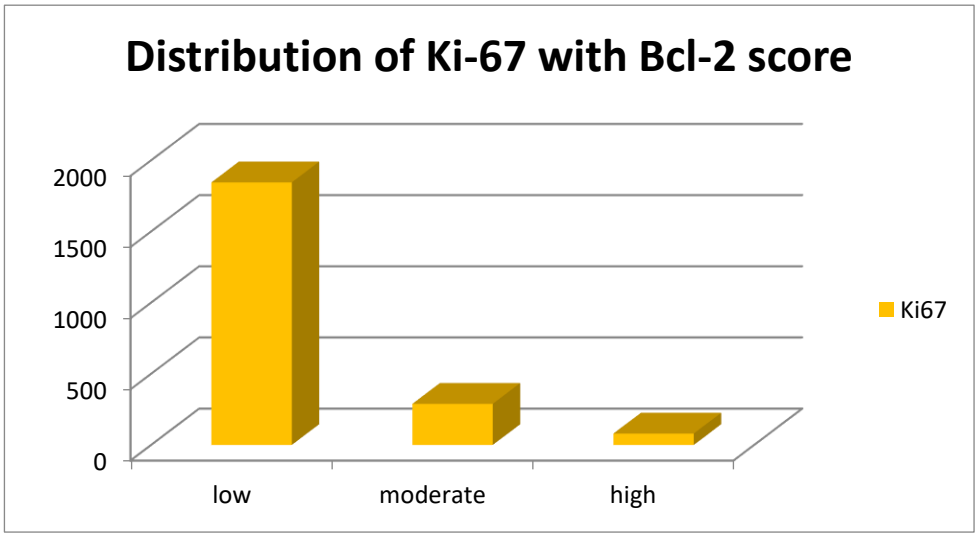

Figure 3: Correlation of Ki-67 and Bcl-2 expression: a strong negative correlation was observed between Ki-67 and Bcl-2 immunoreactivity (r=-0.8896, p<.0001)

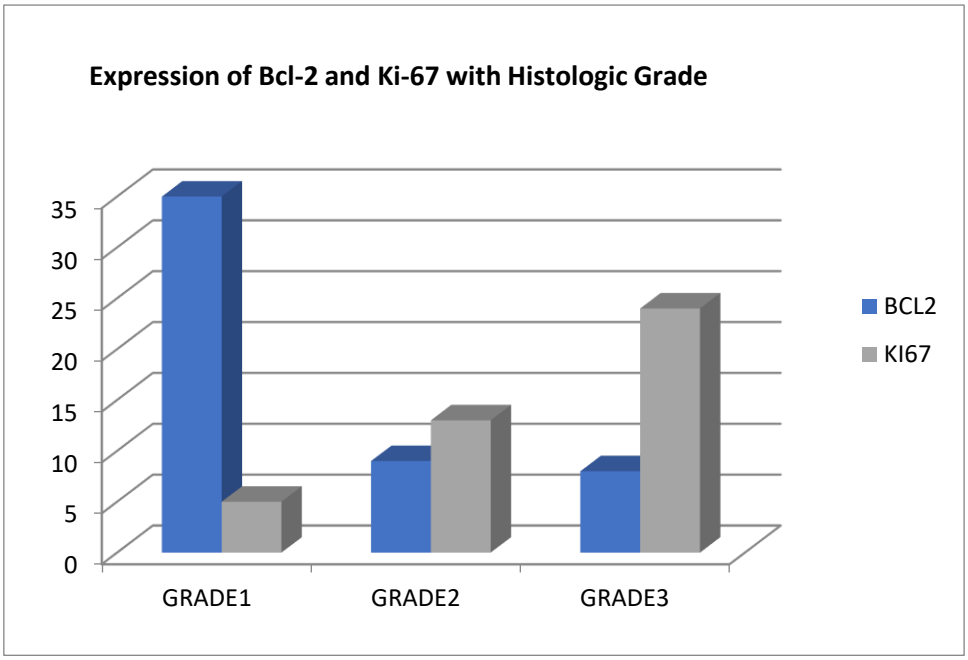

Figure 4: Correlation of Bcl-2 with Ki-67. There is a significant correlation between Bcl-2 and Ki-67 expressions and by Pearsons' correlation coefficient; r= -0.889, p<0.0001

Association between histopathological grade and biomarkers.

Anova test was performed with histopathological grade and biomarkers (Bcl-2 and Ki-67). Significant variations of Ki-67 with grading (f ratio= 141.932, p<0.0001) showed higher percentage of Ki-67 are associated with higher grades. However, Bcl-2 was inversely associated with histological grade (f ratio= 35. 801, p<0.001). (Figure 7-8)

Statistical analysis showed highly significant differences between the Breast carcinoma Grading according to Bcl-2 expression; its rates among breast carcinoma grading showed high percentage of Bcl-2 with Grade 1 (p<0.007947). On the contrary higher Ki-67 expression was associated with Grade 3 carcinoma (p<0.011778). Besides, higher Ki-67 expression was associated with lower Bcl- 2 expression. (Figure 9)

Correlation between all biomarkers and clinical data.

Pearson Correlation Coefficient was used to calculate correlation between histological parameters like Nottingham histologic score with biomarkers like Ki- 67 and Bcl-2. Ki-67 is positively correlated with histological grading (Fig. 2) (r=0.3468, p<0.041) and a strong negative correlation was observed

between Ki- 67 and Bcl-2 immunoreactivity (Fig.10, Fig.11) (r=-0.8896, p<.0001). On the other hand, Bcl-2 was inversely correlated with histological grading (Fig 4) (r= - 0.8983, p<0.001).

Discussion

The expression and correlation of clinicopathological parameters of breast carcinomas with classical biomarkers namely ER, PR, Her2, and Ki-67 have already been established in various studies. In the present study novel marker like Bcl-2 was evaluated and its association with clinicopathological parameters like Histological Grade was studied.

Normal breast development is controlled by a balance between cell proliferation and apoptosis, and there is strong evidence that tumor growth is not just a result of uncontrolled proliferation but also of reduced apoptosis. The balance between proliferation and apoptosis is crucial in determining the overall growth or regression of the tumor. The growth of breast cancer cells is dependent on various viability factors including hormones. The withdrawal of these factors stimulates apoptosis and accordingly, sex steroid receptor-negative tumors had higher apoptotic indices19,20. Apoptosis occurs spontaneously or may be induced by various anti-tumor agents and is regulated by many genes including Bcl-2.

In the present study conducted with 52 breast carcinoma specimens, maximum number of cases belonged to Grade 3 i.e. 34 (65%). Bcl-2 showed a significant association with Histological Grade, showing more Bcl-2 positivity with at lower grades, particularly with Grade 1 i.e. 34 (67%). On the other hand, Bcl-2 was found inversely correlated with Histological Grade, as shown previously (r= -0.8983, p<0.001). Recent studies have shown that Bcl-2 protein is a promising prognostic and predictive marker in Breast Carcinoma, especially in hormone receptor-positive, lymph node negative BC (Ayadi et a, Kallel-Bayoudh et al., 2011; Ali et al., 2012; Paik et al., 2004). Moreover several studies have demonstrated that Bcl-2 is a strong prognostic factor correlated with a better survival (Martinez-Arribas et al., 2007; Dawson et al., 2010; Callagy et al., 2006). In the current study, it was found that Bcl-2 is associated with lower grade and lesser aggressive carcinomas of breast. The numbers of Bcl-2 positive cells were maximum with lower ki-67 expression i.e. 33 (66%). So, an inverse correlation was seen between anti-apoptotic marker Bcl-2 and proliferation index Ki-67. Such correlation was also seen in previously conducted studies by (Ayadi et al, Berardo et al, Kallel-bayoudh et al, Pillai et al). Also, Ki-67 is an important biomarker which provides additional and independent predictive information regarding the response to chemotherapy and the prognosis in a group of patients receiving neoadjuvant treatment. Thus Bcl-2 shows a significant role in further prognosis of breast

carcinoma by expressing with lower proliferative index Ki-67. Though few studies (Lippopen et al, Fabi et al) have shown, higher Bcl-2 expression with higher grade and higher Ki-67 score.

Bcl-2 acts to block apoptosis in vitro and in vivo; thus, elevated levels of Bcl-2 should be correlated with lower apoptosis. Bcl-2 is associated with well differentiated tumors and with lower proliferation index scores as shown in previous studies (Berardo et al).

Ki-67 is an individual predictive marker of breast carcinomas with respect to hormonal receptor positivity, lymph node involvement and response to chemotherapy. Ki-67 can be used to select patients who are unable to benefit from chemotherapy, namely, those Her2-negative and hormone receptor-positive tumors with low proliferation (Fasching et al., 2011; Inwald et al., 2013). In the present study it was established that higher Ki-67 score is associated with higher grades, more aggressive tumor morphology and is also inversely related to prognostic markers like Bcl-2.

Conclusion

The study established that, higher histological grades of breast carcinoma are associated with higher Ki-67 score. Higher Bcl-2 expression was associated with lower grades and lower Ki-67 scores. Thus it can be concluded that Bcl-2 expression is associated with less aggressive tumors and has an inverse relationship with proliferative index Ki-67. These two biomarkers, namely, Ki- 67 and Bcl-2 can be used along with conventional biomarkers to predict the aggressiveness and prognosticate breast carcinoma.

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