

Assessment of Apoptotic Index and Anti-Apoptotic Protein BCL-2 and Their Correlation with Other Histological Prognostic Factors in Breast Cancer

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ABSTRACT

Background: Diseases of breast have been of major concern to the pathologists of all times because of their widespread occurrence. Malignant breast tumors constitute the most common group of cancer among women in several regions of the world. The aim of this study is to determine expression of bcl-2 gene and apoptotic index in breast tumors, to evaluate its implication in prognosis and to correlate it with other histological prognostic factors.

Methods: A histopathological study was carried out in 35 malignant lesions of the breast. Conventional haematoxylin and eosin staining and immunohistochemical staining for bcl-2 oncoprotein were done in all the 35 cases.

Results: A total of 35 malignant breast cases were included in the study. Out of which 15 cases showed bcl-2 positivity (42.86%). There was a significant increase in apoptotic index (A.I.) with the grade of tumor and significant decrease in bcl-2 positivity with tumor grade. No significant correlation was found between tumor size and lymph node status with A.I. and bcl-2 expression. Both A.I. and bcl-2 expression were found to be inversely correlated ($p < .01$).

Conclusion: The statistically significant inverse association between extent of apoptosis and bcl-2 expression suggests that bcl-2 is one of the major regulators of apoptosis in invasive breast lesions.

Key words: Breast Carcinoma, Apoptotic Index, bcl-2.

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Article History:

Received: 04-07-2018, Revised: 27-07-2018, Accepted: 09-08-2018

Access this article online

Website: www.ijmrp.com	Quick Response code 
DOI: 10.21276/ijmrp.2018.4.5.011	

INTRODUCTION

Breast cancer is the most frequent cancer among women, owing to its complex structure and extreme sensitivity to endocrine influences. An estimated 1.67 million new cases diagnosed in 2012 (about 25% of all cancers). Breast cancer ranks as the fifth cause of death from cancer, but it is still the most frequent cause of cancer death in women in developing regions.¹ During 2012, about 144,937 new cases of breast cancer occurred in India, which accounts for 27% of all malignant cases with a mortality of 21.5% of all cancer cases.² The present study is being undertaken as bcl-2 gene and apoptotic index are important prognostic markers and now a days neoadjuvant therapy has become increasingly popular as primary treatment of breast cancer.

AIMS AND OBJECTIVES

1. To quantify apoptosis and obtain apoptotic index in breast cancers.
2. To evaluate Bcl-2 protein expression immunohistochemically in breast cancers.

3. Correlation of apoptotic index and Bcl-2 protein immunoreactivity with known histological prognostic factors in breast carcinomas including- tumor grade, tumor size, and lymph node status.

MATERIALS AND METHODS

The material for the study was mastectomy specimens, as well as excisional biopsies of the breast, sent to the histopathology department over a period of 1.5 years and diagnosed as malignant on histopathology. A total of 35 cases were studied. The specimens were fixed in 10% buffered formal saline, followed by tissue processing, embedding and sectioning. The tissue sections were stained with haematoxylin and eosin. In addition, immunohistochemical staining for Bcl-2 oncoprotein was performed by means of a modified labeled avidin-biotin technique in which a biotinylated secondary antibody forms a complex with peroxidase-conjugated streptavidin molecules using monoclonal antibody and staining kit.

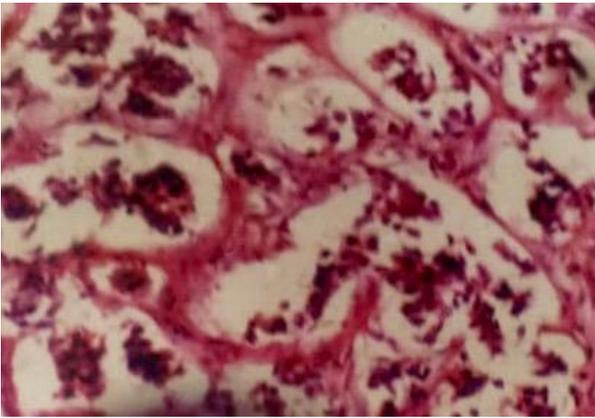


Fig.1a: Ductal carcinoma grade I (H and E, X200)

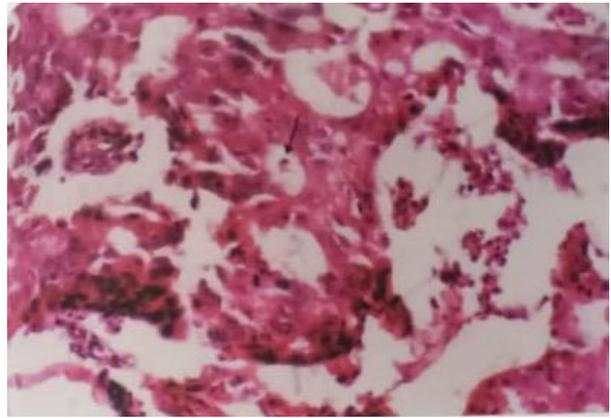


Fig.2a: Apoptosis in grade II ductal carcinoma (H&E, X200)

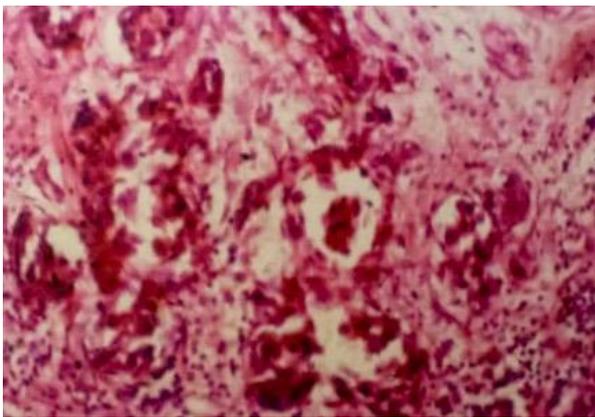


Fig.1b: Ductal carcinoma grade II (H and E, X200)

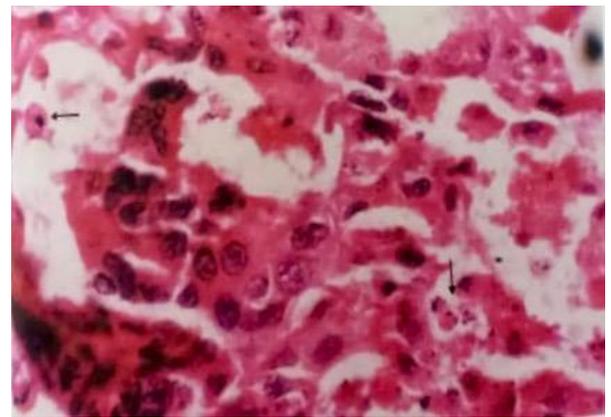


Fig.2b: Apoptosis in grade III ductal carcinoma (H&E, X400)

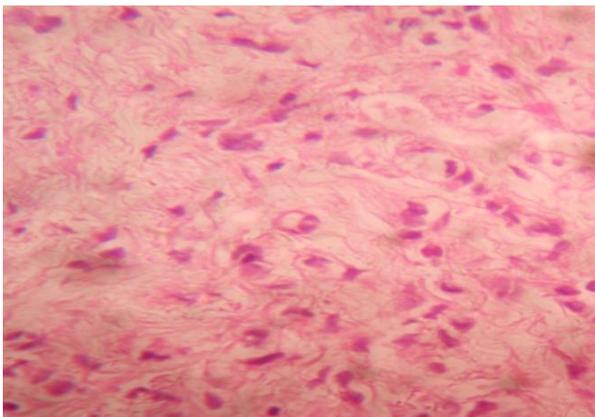


Fig.1c: Lobular carcinoma (H and E, X400)

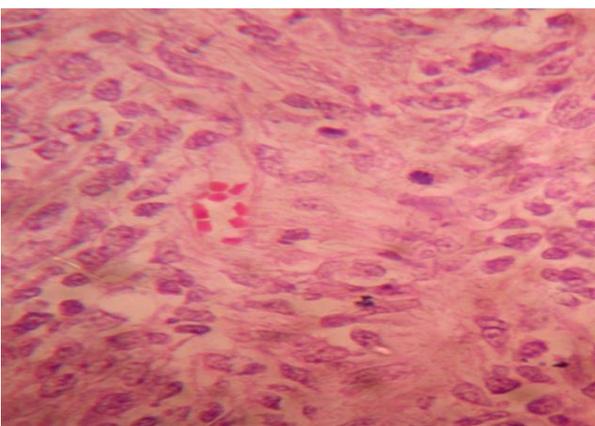


Fig.1d: Malignant phyllodes tumor (H and E, X400)

RESULTS

In our study, 35 cases of malignant breast diseases were included. Out of which 27 cases (77.14%) were of intraductal carcinoma (IDC) (NOS) type, 2 cases (5.71%) were of IDC (small cell type), 3 cases (11.41%) were of IDC with comedocarcinoma, 1 case (2.85%) each of infiltrating lobular carcinoma and malignant phyllodes tumor. [Figure.1a-d]. Age group involved in our series was 31-60 years. Maximum number of cases (24 out of 35) were in the age group of 31-50 years, forming 68.60% of the total cases.

The invasive breast lesions were graded according to the Nottingham modification of Bloom-Richardson system. Out of 35 cases, 10 cases (28.57%) were of grade 1, 15 cases (42.86%) were of grade 2 and 10 cases (28.57%) were of grade 3. The tumors were graded according to their size into 3 categories. Total number of cases of tumor size 0-2 cm were 3 (8.57%), 12 cases (34.29%) were in the range of size 2.1-5 cm, while 20 cases (57.14%) were >5 cm in size.

Total number of cases with no positive lymph node were 7 (20%), cases with 1-3 positive lymph nodes were 10 (28.57%) and cases with >3 positive lymph nodes were 18 (51.43%).

Apoptotic index (A.I.) was calculated in all the H & E stained sections in 1000 tumor cells, showing maximum apoptosis and mean A.I. was calculated in different grades, different tumor size and lymph node (L.N.) status of breast carcinoma. [Figure.2a and b]. A.I. of grade I and II tumor and grade I and III tumor, were compared, and found to be statistically significant ($p < .01$). On comparing A.I. of grade II and III tumor, it was found to be statistically significant ($p < .05$). [Table. 1a]. Cases with tumor size

0-2 cm had mean A.I. 3.67, 2.1- 5 cm had 5.67 and >5 cm sized tumors had 3.0. The association of A.I. with tumor size was found to be statistically insignificant ($p>.05$). [Table.1b].

Cases with 0 L.N. had A.I. of range 2-8 with mean (3.85, 1-3 positive L.N. had 1-10 A.I., with mean 4.55 and >3 L.N. had 0-8 A.I. with mean 3.53. The association of A.I. to lymph node status was found to be statistically insignificant ($p>.05$). [Table. 1c]

Table. 1a: Apoptotic index in Grade I, II, and III lesions

Grade	No. of cases (n)	Range of A.I.	Mean	SD
Grade-I	10	0-5	1.85	1.305
Grade-II	15	2-8	4.13	2.101
Grade-III	10	4-10	6.5	3.324

Table. 1b: Apoptotic index in different tumor size

Tumor size	Range of A.I.	Mean	SD
0-2 cm	0-5	3.67	1.7
2.1-5 cm	2-8	5.67	2.75
>5 cm	4-10	3.0	2.47

Table. 1c: Apoptotic index and lymph node status

L.N. status	Range of A.I.	Mean	SD
0	2-8	3.85	2.295
1-3	1-10	4.55	3.609
>3	0-8	3.53	2.245

Table.2a: bcl-2 positivity in grade I, II and III lesions

Grade	No. of cases	Range of bcl-2 positivity	Mean	SD
I	10	0-9	6.20	3.655
II	15	0-9	2.87	3.827
III	10	0-6	0.30	0.458

Table.2b: bcl-2 positivity and tumor size

Tumor size	Range of bcl-2 positivity	Mean	SD
0-2 cm	0-9	3.00	4.243
2.1-5 cm	0-9	1.75	2.861
>5 cm	0-9	2.81	3.673

Table.2c: bcl-2 positivity and lymph node status

L.N. status	Range of bcl-2 positivity	Mean	SD
0	0-1	1.0	0.926
1-3	1-9	2.40	2.615
>3	0-9	1.39	2.475

Table 3a: Relation of AI & bcl-2 positivity with tumor grade

Grade	Mean A.I.	Mean bcl-2 score	SD
I	1.85	3.70	4.234
II	4.13	1.47	3.436
III	5.90	0.80	2.446

Table.3b: Relation of A.I. and bcl-2 positivity with tumor size

Tumor size	Mean A.I.	Mean bcl-2 score
0-2 cm	3.67	3.0
2.1-5 cm	5.67	1.75
>5 cm	3.0	2.81

Table.3c: Relation of AI & bcl-2 positivity with lymph node status

L.N. status	Mean A.I.	Mean bcl-2 score
0	3.85	1.0
1-3	4.55	2.40
>3	3.53	1.39

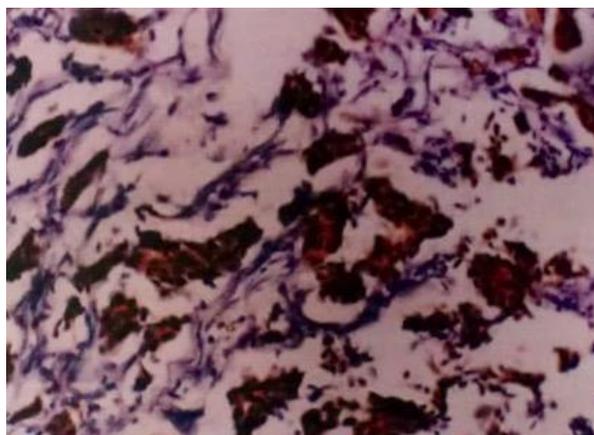


Fig.3a: Intense Bcl-2 positivity in grade I ductal carcinoma (x200)

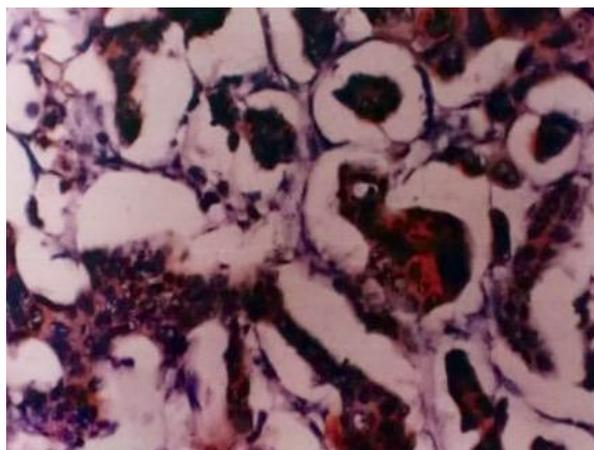


Fig.3b: Moderate Bcl-2 positivity in grade II ductal carcinoma of (X400)

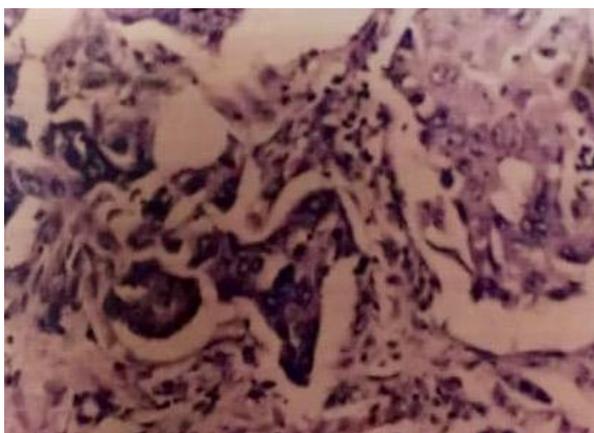


Fig.3c: Negative Bcl-2 staining in grade III ductal carcinoma (X400)

Out of 35 cases, 15 cases were considered bcl-2 immunopositive (42.86%). Low expression was seen in 7 cases (20%), moderate expression was seen in 3 cases (8.57%), and strong expression was seen in 5 cases (14.29%). Grade I lesions had mean bcl-2 positivity of 6.20, grade II lesions had mean bcl-2 positivity of 2.87, while grade III lesions had mean bcl-2 positivity of .30.[Figure 3a-c]. The decrease in bcl-2 positivity from grade I to grade II and from grade II to grade III were statistically significant ($p<.05$). [Table.2a].

Bcl-2 positivity in cases with tumor size 0-2 cm had mean bcl-2 positivity 3.00, 2.1-5 cm size cases had 1.75 positivity and >5 cm sized tumors had 2.81 positivity. The association of bcl-2 positivity with tumor size was found to be statistically insignificant ($p>.05$). [Table.2b] Cases with 0 L.N. had mean bcl-2 positivity of 1.0, cases with 1-3 L.N. had 2.40 and cases with >3 L.N. had mean value of 1.39. The correlation of bcl-2 positivity to the lymph node status was statistically insignificant ($p>.05$). [Table.2c].

There was an increase in A.I. and decrease in bcl-2 positivity with the grade and this association between bcl-2 and A.I. was found to be highly significant ($p<0.01$). [Table. 3a]. No correlation of A.I. and bcl-2 positivity with tumor size was found. [Table. 3b]. Also there was no correlation of A.I. and bcl-2 positivity with lymph node status.[Table.3c].

DISCUSSION

Diseases of the breast constitute the great majority of the causes of morbidity and mortality worldwide. Thus, the breast pathologies deserve the preferential attention of the doctor. Large number and variety of papers published on carcinoma of breast, prognosis and associated factors are an index of the complexity of the problem and indeed the study of tumor.

Apoptosis is a physiological process following which normal cells die after a given number of replications. Tumor cells tend to interfere with this mechanism by activating genes which inhibit apoptosis, thus, achieving long term survival or even immortality, as shown in experimental in vitro models.^{3,4} One of the main genes limiting apoptosis is Bcl-2. Paradoxically, Bcl-2 expression has been consistently associated with a better prognosis of breast cancer patients, when, from a purely theoretical point of view, quite the contrary should be expected.^{3,4} Most studies of Bcl-2 expression in breast cancer have been carried out using immunohistochemical techniques.⁵⁻⁷

In the present study, 35 cases of malignant breast lesions were included. All 35 cases were of invasive carcinoma. All invasive epithelial tumors were graded according to Nottingham modification of Bloom and Richardson. According to Elston CW and Ellis IO,⁸ who had modified Bloom and Richardson method through Nottingham/Tenovus primary breast cancer study, histological grading is directed principally at invasive adenocarcinoma; tumors of other types are not suitable for the particular method they describe.

In our study, there was significant increase in A.I. from Grade-I to Grade-II ($P<.01$) and from Grade II to Grade III 9p ($p<.05$). Our study was comparable to the study of Lipponen P⁹ and Villar E et al,¹⁰ they found that high apoptotic index was associated with increasing grade of the tumor. In our study, the association of A.I. with tumor size was not statistically significant ($p>.05$). Lipponen P⁹ and Berardo et al¹¹ had also found no correlation between A.I. and tumor size in their studies. While Zhang et al¹² in their study reported, that large tumors had significantly higher A.I. than small tumors.

There was no significant association between A.I. and lymphnode status ($p>.05$) in our study. This finding was consistent with the study by Lipponen P⁹, who concluded that A.I. is not correlated to axillary L.N. status ($p>.05$). However Berardo M et al¹¹ found that high A.I. was positively correlated with the number of involved nodes, but the relationship was only of borderline significance ($p=.06$).

In our study, bcl-2 immunostaining was done in all the 35 cases, to find relationship of A.I. with other histological parameters. A total of 15 cases were considered bcl-2 positive. There was a significant decrease in bcl-2 positivity from Grade-I to Grade III lesions. Honma N et al¹³ and Martinez-Arribas F et al,¹⁴ in their studies, had also found the same correlation between bcl-2 positivity and tumor grade. In our study, no significant correlation was found between bcl-2 positivity and tumor size. It was comparable to the study performed by Leek RD et al,¹⁵ found that no relationship could be observed between bcl-2 expression and tumor size. However, in the studies performed by Honma N et al¹³ and Martinez-Arribas F et al,¹⁴ bcl-2 expression was significantly correlated with smaller tumor size.

In our study, the association between bcl-2 positivity and lymph node status was not statistically significant ($p>.05$). Leek RD et al,¹⁵ in their study found that bcl-2 expression did not correlate with lymph node status. However, in the study by Berardo M et al,¹¹ there was a significant correlation of bcl-2 expression with lymph node status ($p=0.02$).

In our study, we found a negative correlation between apoptotic index and bcl-2 positivity ($p<.01$). Our study was comparable to the study of Mustonen M et al¹⁶ and Lipponen P,⁹ found that intensity of bcl-2 expression was inversely related to apoptotic index. From this study, it is postulated that there is gradual increase in the extent of apoptosis and a decrease in bcl-2 expression in breast lesions as they become histologically more aggressive. Although the number of cases studied is small and long follow up is necessary.

CONCLUSION

To conclude, besides histological grade and axillary node status, the established prognostic markers-apoptotic index and bcl-2 oncoprotein expression can be used as an independent prognostic markers, but further studies and long follow up of the patients are needed to confirm the results of the study and establish them as prognostic factors in breast carcinoma.

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Source of Support: Nil. **Conflict of Interest:** None Declared.

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Cite this article as: Meenakshi Pathak, Chaitali Gupta, Kalpana Singh. Assessment of Apoptotic Index and Anti-Apoptotic Protein BCL-2 and Their Correlation with Other Histological Prognostic Factors in Breast Cancer. *Int J Med Res Prof.* 2018 Sept; 4(5):46-50. DOI:10.21276/ijmrp.2018.4.5.011